Evolution in a Heterogeneous Environment

A Thesis presented for the degree of Doctor of Philosophy

of the University of Stirling

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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

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Abstract

This thesis questions whether sympatric divergence, brought about by disruptive selection in a spatially heterogeneous environment, can occur under natural conditions and in the laboratory.

I. An investigation, to detect micro-differentiation, was made on a Plantago lanceolata population, comprising a gradation of phenotypes and occurring in a small area (1 x 5m) of dune pasture, heterogeneous by virtue of different vegetation heights. The prostrate phenotype with shorter leaves and inflorescences was correlated with low vegetation; the erect phenotype was correlated with taller vegetation. Vegetative propagules in standard conditions of garden and greenhouse showed persistent genotype differences of growth habit, leaf length and inflorescence length. Growth habit and leaf length also correlated with the original environment, indicating adaptive micro-differentiation. Some phenotypic plasticity was apparent. Investigation of the field population revealed flowering time differences between the phenotypes and rapid turnover of individuals less than six months old, particularly in low vegetation where the Plantago population was the most dense. Selection pressures appeared to be operating to maintain differentiation within this heterogeneous environment. Population control was evident, with mortality matching recruitment, but the chances of survival of an individual were independent of the season of establishment.

II. In a second series of experiments, a population of randomly-mating Drosophila melanogaster was maintained for 20 generations in small 'population cages', heterogeneous because they contained two types of food medium, viz. normal food and normal food plus peppermint essence. The founder population yielded 40% more progeny

on the normal food. There were three control populations feeding on (1) normal food only, (2) peppermint food only, (3) homogeneous half-strength peppermint only. The experimental population initially responded to the heterogeneous environment (with its choice of food media), by yielding numbers of progeny and biomass in excess of expectation, which was calculated from the controls. This was thought to be an environmental response. The difference between the observed yield and expectation increased steadily for 10 generations, implying adaptation to the heterogeneity, but, after 17 generations of selection, the yield was significantly less than expectation. This persisted for a generation of lapsed selection on normal food, indicating a genetic response to some factor within the heterogeneous environment. Because females reared on peppermint showed a behavioural change and tended to choose this less palatable medium on which to lay their eggs, it is suggested that a genetic component of behavioural flexibility contributed to this result. There was also evidence of improved adaptation to normal food, possibly a genetic response to highly competitive conditions on this densely-populated medium. Although sympatric divergence was not conclusively demonstrated, a measure of habitat selection for egg-laying sites developed and the population became non-random. Peppermint retards the life-cycle of the flies living on it by approximately one day. Therefore, the heterogeneous population was experiencing conditions which might promote assortative mating.

It was concluded from the two experiments, that a heterogeneous environment may act disruptively on a small, randomly-breeding population within a small area. The Plantago population, in an environment where selection pressures were probably high, showed evidence of micro-differentiation, indicating that sympatric

divergence had occurred, although phenotypic plasticity was also evident in some morphological characters. The Drosophila population, in a heterogeneous environment where selection pressures may have been relatively low, also became non-random and evolved habitat-choice. In both investigations, forces enhancing assortative mating, helping to maintain genetic variation by reducing gene flow, were apparent. Therefore, it is concluded that sympatric divergence may be brought about by disruptive selection in a heterogeneous environment.

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SECTION I

GENERAL INTRODUCTION

General introduction

'Another lack is in Darwin's failure quite to grasp the roles of populations and their isolation in speciation'.

George Gaylord Simpson in Foreword to 'Origin of Species'

The mechanism of evolution, whereby variant types arise and diverge from common ancestry by a process of natural selection, has fascinated many biologists since Darwin first propounded his theory in 1858. For evolutionary progress to be made, directional forces of natural selection brought about by the environment, act on variants within the evolutionary units, which Darwin regarded as the species and which today are commonly regarded as the populations comprising the species. But most environments are heterogeneous, with the result that selective forces, even if directional in an overall sense, may act disruptively on the inhabitant species or populations.

Darwin and early twentieth century scientists considered evolutionary aspects on a grand scale. Spatial environmental heterogeneities were thought of in terms of hundreds of miles. Gross morphological differences between species were studied and the time scale involved in such changes was of the order of millions of years. It was not appreciated that evolution and genetic change on a smaller scale were sufficiently rapid to be detected.

Today, evolutionary studies are pursued at a more modest level and the dynamic interactions of populations and their environments are being emphasized. The effects of both temporal and spatial heterogeneity of the environment on the genetic

structure of populations are being investigated. In a spatially heterogeneous environment, however, as the distance between the diverging populations being studied becomes less, so the extent of gene flow between the populations becomes increasingly important. It was thought that gene flow between different populations, or between individuals within the populations, would tend to prevent any divergence being brought about by disruptive selection. Before divergence could occur, it was thought that isolation must exist between different populations or between pockets of individuals within a population. Mayr (1963) held the view that geographical isolation was a pre-requisite to reproductive isolation and subsequent speciation (allopatric speciation). Although allopatry is known to be wide-spread (see Cain 1954, Mayr 1963), the feasibility of sympatric speciation (speciation despite gene flow) is becoming increasingly recognised. This process can be thought of as occurring in three steps: 1) sympatric divergence, whereby disruptive selection pressures in a heterogeneous environment give rise to polymorphism; 2) the origin of reproductive isolation between the divergent types; 3) complete reproductive isolation and sympatric speciation.

Levene (1953) considered the first of these steps. He showed, mathematically, that it would be possible to create a polymorphism and maintain the variants in a population if the population was assumed to be split into sub-populations, each inhabiting a different niche in the environment and each niche independently controlling the numbers of its sub-population. Mating was assumed to be random over the entire population and was followed by a random redistribution of the population among the niches. This

model was subsequently modified by Deakin (1966) and Prout (1968) who obtained similar results to those of Levene when, more realistically, the conditions allowed any pattern of migration between the niches rather than a random redistribution at every generation. Other models, concerned with the fate of a gene in a habitat with two niches, have been proposed by Parsons (1963), Hanson (1966), Jain & Bradshaw (1966), Levins & McArthur (1966), Antonovics (1968a & b) and Smith (1970). They all demonstrate the theoretical feasibility of sympatric divergence. Probably the most relevant and exhaustive model was that proposed by Smith (1966). He confirmed Levene's model but, in addition, found a condition for a stable polymorphism in a two-niche situation when there is dominance. He discussed the likelihood of sympatric speciation occurring after the establishment of a polymorphism by this method and decided that it could well be possible because reproductive isolation might well arise by habitat selection, reinforced by genes causing assortative mating.

These models, however, are all concerned with the inheritance of discontinuous characters. According to Mather (1955), disruptive selection could give rise to a bimodality in a continuously varying population, provided that the selection pressures acting on the extreme variants could be maintained independently. Dickinson & Antonovics (1973a) in their theoretical considerations of sympatric divergence using computer simulation, show that the results of niche models for polygenically-inherited characters are similar to those for single gene inheritance but slower, namely, sympatric divergence is possible provided that the disruptive selection pressures are high, consistent and

balance the gene flow. They further develop their model to show that full speciation can eventually occur given sufficiently strong selection pressures and an opportunity for assortative mating, especially selfing in plants.

Theoretically, therefore, it has been adequately demonstrated that sympatric divergence, followed by reproductive isolation and speciation, is feasible.

This thesis questions whether sympatric divergence, brought about by disruptive selection in a spatially heterogeneous environment, can occur under natural conditions and in the laboratory. Section II describes an investigation to find out whether the genotypic and phenotypic structure of a natural population of Plantago lanceolata is consistent with the phenomenon of sympatric divergence in a heterogeneous environment. Section III describes an attempt to demonstrate sympatric divergence in a population of Drosophila melanogaster in a model situation in the laboratory. The literature on field and laboratory investigations is reviewed in the introductions to sections II and III respectively.

SECTION II

PLANTAGO LANCEOLATA IN A HETEROGENEOUS ENVIRONMENT

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"The book begins with Plantago, plantain, 'because it is common and because more than any other plant it bears witness to God's omnipotence'" - E. Nordenskjold (History of Biology, 1928) Quoting from Herbarum Vivae Eicones, Otto Brünfels, 1488-1534.

1. Introduction

It was Turesson (1925) who first demonstrated the existence of ecotypes, plants that were genetically adapted to their environments and not merely tolerant of them. Theoretical studies, particularly that of Smith (1966) (see Section I, p 3), and the work of Thoday et al (see Section III, p 105), which contradicted earlier ideas that genetic differentiation would be reduced, if not eliminated, by the swamping effects of gene flow, stimulated the study of differences between ecotypes situated only a short distance apart. There was considerable evidence of differentiation in parapatric situations (populations in different locations but between which there is considerable gene flow) before 1972. This was demonstrated most clearly between populations at abrupt boundaries by the work of Bradshaw and his associates (Jain & Bradshaw 1966, Aston & Bradshaw 1966, McNeilly 1968, McNeilly & Antonovics 1968, Antonovics & Bradshaw 1970). Gradual changes in the environment had already been shown to be reflected in clinal patterns of population differentiation, as in P. maritima (Gregor 1930). Snaydon (1970) demonstrated small-scale clinal differentiation in Anthoxanthum odoratum. Bradshaw (1972) argued the feasibility of small-scale micro-differentiation but prior experimental evidence of such was scant (Bradshaw, McNeilly & Gregory 1965, Snaydon 1970). Dickinson & Antonovics (1973a & b), extending Smith's theory (1966) to conditions likely to apply to plant populations in a heterogeneous environment, showed that in a polymorphic population there may be a precise match of the morph:

with the patchiness of the environment. The most convincing evidence of such adaptation until this time, came from the work of Snaydon (reported in Bradshaw, McNeilly & Gregory 1965). He showed that plants of Agrostis canina and Festuca ovina growing directly below a galvanised (zinc coated) fence were tolerant to enhanced levels of the metal, whereas plants a few inches away from the fence were not. Snaydon (1970) also showed that a distance of less than 150mm separated contrasting populations of Anthoxanthum odoratum growing on soils given different fertilizer treatments over a period of 50 years. Further investigations on this site (Snaydon & Davies 1972, Davies & Snaydon 1976 and Snaydon & Davies 1976), which were published after the work in this thesis was completed but before its publication, have since presented evidence that morphologically different populations of A. odoratum are genetically different, precisely matched to the environment, and that partial reproductive isolation exists between them.

An investigation of micro-differentiation was therefore carried out on a small, natural population in 1969. The study differs in two important aspects from previous work. Firstly, previous studies, particularly on adjacent populations, had frequently dealt with discrete boundaries between relatively extreme environments, such as mine and pasture (McNeilly 1968; McNeilly & Antonovics 1968; Antonovics & Bradshaw 1970) or exposed cliffs and streams (Aston & Bradshaw 1966): the present study was therefore centred on a relatively 'normal' pasture in which the main obvious heterogeneity was vegetation height, such as is common in pasture habitats. Secondly, it was also decided to look for micro-differentiation on a very much smaller scale than

previously, more specifically, within an area where gene exchange and seed dispersal might be essentially random. The study area chosen was 1 metre by 5 metres in extent (p 30).

Plantago lanceolata was chosen as the experimental plant. The existence of different phenotypes is a well-documented phenomenon in the Plantaginaceae: for P. lanceolata (Jenkin 1925, Pilger 1937, Böcher 1943, Sagar & Harper 1964), for P. media and P. major (Sagar & Harper 1964) and for P. maritima (Gregor 1938). P. lanceolata was shown to be genetically very variable by Jenkin (1925), Pilger (1937) and Böcher (1943). It grows in a wide variety of habitats and, compared with P. media and P. major, small populations comprising different phenotypes may more readily be found. The most obvious phenotypic difference is in the growth habit of the leaves and spikes, which may be prostrate, erect or intermediate between the two positions. P. lanceolata is a rosette plant and can be precisely located to a specific position in the habitat: this is essential when looking for differences over very short distances. It is a perennial that can be readily cloned and transplanted so that genotypes can be replicated and studied under standard garden or greenhouse conditions: sampling as adults avoids losing resolution as a result of the segregation that would occur in a seed sample.

A preliminary investigation of 5 wild populations, each of predominantly one phenotype of P. lanceolata from geographically different areas, was undertaken to familiarise the experimenter with the morphology of the plant and to develop suitable cloning and transplanting techniques. A further preliminary experiment was carried out on the two populations of the five which were the most different from each other in growth habit. This was to

determine whether the growth habit of the plants could be altered by shading (a relatively obvious environmental parameter) thus demonstrating phenotypic flexibility. If phenotypic flexibility were found, then it was important to know whether this would mask genetic differences between the populations. This would be of direct relevance to the main investigation in which different phenotypes of a single population were to be removed to standard cultivation conditions for later comparison.

Table 1. Location and description of Plantago populations

Habitat	Location	General Appearance
1. Exposed rocks near sea	Penmon Point, Anglesey*	Very prostrate, leaves often reflexed
2. Zinc mine, on tailings	Trelogan, Flintshire*	Prostrate plants, sparse cover
3. Zinc mine, in grassland near mine boundary c. 100m from Population 2	Trelogan, Flintshire*	Plants erect among tall grass, mainly *Agrostis stolonifera*
4. Waste ground; overgrown garden	Menai Bridge, Anglesey*	Plants erect
5. Commercial seed	France, supplier unrecorded	Unknown: probably erect pasture type

* North Wales, U.K.

2. Study of Contrasting Populations of P. lanceolata

2:1 Morphology

Methods

Seeds were collected from 5 contrasting populations of P. lanceolata, each population comprising predominantly one phenotype as assessed by growth habit. One was a commercial population from France, and the others collected by J. Antonovics in 1967 from contrasting habitats (table 1). 28 seeds from each population were sown at 5cm spacing and 0.5cm deep in trays 37cm x 23cm: there were 2 trays per population and the trays were completely randomised. The seeds were grown on John Innes No. 1 potting compost and kept in a heated greenhouse, without artificial light, for 6 months. In order to compare the morphology of the plants, the following measurements were taken:

1. Seed germination: the number germinating each day,
2. after 1 month: a) number of leaves
   b) length of longest leaf
   c) growth habit*
   d) number of inflorescences,
3. after 6 months: a) number of leaves
   b) length of longest leaf
   c) growth habit*
   d) width of widest leaf.

*Growth habit was noted on several occasions at weekly intervals and a score of 1 given for prostrate habit (the leaves pressed flat against the substrate), 2 for intermediate growth habit (the leaves subtending an angle of approximately 45° to the vertical) and 3 for erect growth habit (leaves vertical);

the growth habit score averaged over the 1 month (or 6 months) was then used as a measure of growth habit for the statistical analysis. This frequency of measuring was adopted because it became apparent that small fluctuations of temperature and humidity slightly affected the growth habit of the plants in the greenhouse.

The number of inflorescences was not measured after 1 month. So few plants flowered during the experiment due to the high density of sowing, that this character did not provide a good basis for comparison. For this reason it was not used in the correlation analysis after 1 month's growth. It also became apparent, later in the experiment, that the width of the leaf was a key character in the description of the phenotypes. Therefore, this was measured at 6 months.

The germination results were used to give the final percentage of germinated seeds and the 50% germination figures for the 5 populations. The measurements taken after 1 month's and 6 months' growth were analysed firstly, by Duncan's Multiple Range Test on the means of the characters to detect population differences (table 2, p 17), and secondly, by correlation of the characters with each other, within each population, to see whether distinct Plantago phenotypes were evident (table 3, p 19).

Results

i) Germination

Population 2 differed from the rest in that the final percentage of germinated seeds was 97% compared with 82% in population 5 and 84% in the other three populations (fig. 1; Appendix 1, table 1, p 192). The seeds in population 2 also germinated significantly more quickly (50% of seed sown in <4

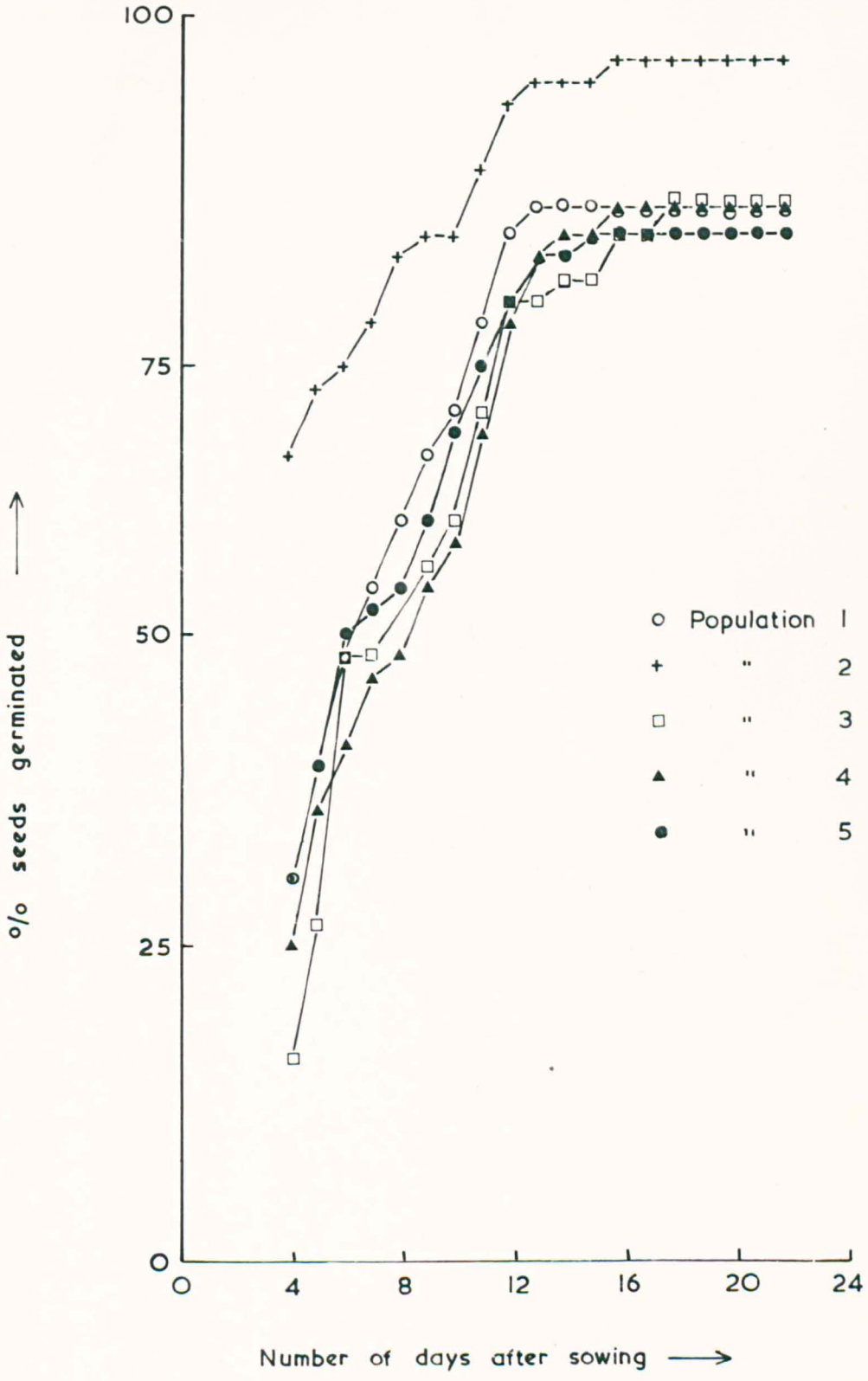


Fig. 1 Germination rate of 5 populations of P. lanceolata (standard errors in table 1, appendix 1, p 192).

Table 2 Morphology of contrasting Plantago populations

a) After 1 month's growth

Population	No. Plants	Character: Leaf no. $\bar{x}$	s.e.	sign.*	Leaf length (cm) $\bar{x}$	s.e.	sign.*	Growth habit $\bar{x}$	s.e.	sign.*	Inflo. no. $\bar{x}$	s.e.	sign.*
1	47	8.60	±0.23	a	7.10	±0.25	a	0.21	±0.15	a	0.23	±0.11	b
2	51	8.18	±0.23	a	10.84	±0.36	c	1.10	±0.19	b	0.27	±0.13	b
3	47	6.53	±0.26	b	9.49	±0.38	b	2.49	±0.12	c	0		b
4	45	6.60	±0.25	b	9.88	±0.39	bc	2.36	±0.15	c	0.13	±0.08	b
5	45	6.87	±0.17	b	10.94	±0.39	c	2.40	±0.15	c	0.96	±0.17	a

b) After 6 months' growth

Population	No. Plants	Character: Leaf no. $\bar{x}$	s.e.	sign.*	Leaf length (cm) $\bar{x}$	s.e.	sign.*	Growth habit $\bar{x}$	s.e.	sign.*	Leaf width (cm) $\bar{x}$	s.e.	sign.*
1	23	8.09	±0.42	bc	39.65	±0.97	b	1.74	±0.15	a	2.48	±0.16	a
2	27	10.04	±0.70	a	40.32	±1.26	b	2.52	±0.11	b	2.24	±0.11	a
3	25	7.44	±0.63	bc	40.68	±1.29	b	2.64	±0.13	b	1.84	±0.12	a
4	23	8.74	±0.56	ab	40.17	±1.33	b	2.61	±0.14	b	1.98	±0.10	a
5	22	6.86	±0.51	c	34.51	±1.41	a	2.59	±0.11	b	1.93	±0.09	a

Growth habit: scored on a 1 - 3 scale; 1 = prostrate growth habit
2 = intermediate growth habit
3 = erect growth habit

$\bar{x}$ = mean value

s.e. = standard error

sign.*: Duncan's Multiple Range Test; for each character, populations labelled with the same letter are not significantly different at the 5% level but are significantly different from populations labelled with different letters.

days) than any of the other seed samples (50% in 6 days for population 5, 7 days for population 1 and 9 days for populations 3 and 4). It was noted that the seeds which were initially the fastest to germinate came from a prostrate population and that, at first, the greatest differences in germination were observed between populations 2 and 3, predominantly prostrate and erect populations, which were situated only 100m distant from each other in the field.

ii) Morphology after 1 month

If the mean values of the morphological characters measured for the 5 populations are compared (table 2; Appendix 1, table 2, p 193), it can be seen that populations 3 and 4 are similar. The plants comprising these populations were erect in growth habit, had 6.5 leaves which were 9.5cm long on average, and had no inflorescences. Of the other 3 populations, population 5 most closely resembled populations 3 and 4, differing in that there was approximately 1 inflorescence per plant. On average, the leaves of population 5 were longer (11cm) but not significantly so. This population was also erect in growth habit. Populations 1 and 2 differed markedly from populations 3, 4 and 5 and, to some extent, from each other. Population 1 comprised very prostrate plants with a large number of short leaves and with one plant in four producing an inflorescence. The plants in population 2 were also prostrate, though not quite as reflexed as those in population 1. They had a similar number of leaves, which were much longer than those of population 1 and comparable to those of population 5. They had a similar number of inflorescences to plants in population 1. The results were analysed by Duncan's Multiple Range Test.

Table 3. Character correlations of contrasting Plantago populations

a) After 1 month's growth

Population	Correlation of leaf no. with: leaf length[1]	growth habit[2]	Corr[n.] of leaf length with growth habit[2]	
1	0.317	-0.689	-0.232	(Corr[n] coeff.)
	*	***		(significance)
2	0.490	-0.579	-0.137	
	***	***		
3	0.530	-0.496	0.034	
	***	***		
4	0.721	-0.458	-0.330	
	***	***	*	
5	0.493	-0.448	-0.100	
	***	**		

b) After 6 months' growth

Population	Correlation of leaf no. with: leaf length[1]	growth habit[2]	leaf width[1]	Corr[n.] leaf length & growth habit[2]	leaf width[1]	Corr[n.] growth habit with leaf width[2]
1	0.065	-0.396	0.488	-0.247	0.257	-0.246
			*			
2	0.428	-0.309	0.719	-0.099	0.407	-0.340
	*		***		*	
3	0.369	-0.337	0.706	-0.243	0.746	-0.233
			***		***	
4	0.292	-0.665	0.462	-0.103	0.354	-0.529
			*			**
5	0.238	0.376	0.171	0.401	0.011	-0.482
						*

Key: 1 - Pearson-moment correlation
2 - Spearman Rank correlation

Levels of significance: * $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

It is noticeable that the seedling populations 1, 2, 3 and 4, closely resembled their parental populations in growth habit. The parental growth habit of the commercial seed was not known but presumably would be erect. P. lanceolata, being rich in minerals, is occasionally sown in pasture to give supplementary feeding to sheep.

When the morphological characters within each population were correlated (table 3a), it was found that as the number of leaves increased, leaf length also increased and the growth habit became more prostrate, regardless of the population from which the plants came. Leaf length was not directly correlated with growth habit, however, except in population 4.

iii) Morphology after 6 months

After 6 months' growth (table 2b; Appendix 1, table 3, p 194), the differences between the 5 populations were less noticeable than at one month. Populations 3 and 4 were still similar to one another. The plants were erect and had approximately 7 - 9 leaves, which were comparatively long (40cm) and narrow (width approximately 1.9cm). The plants in population 5 were also erect and had a similar number of leaves to those in populations 3 and 4, but the leaves were much shorter (approximately 34cm) though of similar width (1.9cm). The plants in population 2 became as erect in growth habit as those in populations 3, 4 and 5 although they had more leaves (approximately 10). The leaves in all the populations (except 5) were of similar length and width. The plants in population 1 were still more prostrate in growth habit than those in the other populations but, in other respects, the plants were similar to the plants in populations 3 and 4.

After 6 months, there were fewer significant correlations between the characters within the populations (table 3b). In all the populations (except population 5), the more leaves a plant had, the wider they tended to be. In two of the more erect populations, 4 and 5, plants with wider leaves tended to be more prostrate in growth habit.

Discussion

It was found that after one month's growth in the greenhouse there was a correlation between leaf number and growth habit, the more prostrate plants having more leaves. This was found in all five populations. Seed collected from a prostrate field population also germinated more rapidly than that collected from an erect field population. One can therefore distinguish a gradation of phenotypes, those at the one extreme having more leaves, prostrate growth habit and more rapid germination and those at the other extreme having fewer leaves, erect growth habit and slower germination. These differences were assumed to be genetic since they were obtained from seed grown under similar environmental conditions and the growth habit of the different populations reflected the growth habit of the respective field populations from which the seeds were collected. Although P. lanceolata is 99% self-sterile (Sagar & Harper 1964) and wind-pollinated, it was assumed that the male parent would be a member of the same population as the mother plant because large, uniform and discrete populations were chosen. Highly significant population differences were found, with the exception of populations 3 and 4 which were from similar, although widely separated, habitats.

That discrete populations of plantains from different habitats may be genotypically and phenotypically different is not a surprising result. From logical considerations and from Turesson's conclusions (1925) that plants are genetically adapted to their environment and not merely tolerant of it, it may be supposed that in exposed situations, such as mine tailings (population 2) or cliffs exposed to high winds (population 1), the prostrate form with more rapid germination would have a selective advantage over the erect form with less rapid establishment. In tall vegetation, from which populations 3 and 4 were collected, the erect form would have a selective advantage over the prostrate form. Indeed, Gregor (1938) found distinct phenotypic differences between populations of P. maritima growing in different habitats, the more prostrate form being found in the more exposed situations. In discrete habitats such as those of populations 1, 4 and 5 investigated above, there would probably be little gene flow between the populations tending to swamp their genetic divergence. The two mine populations, 2 and 3, however, were only 100m apart. Gene flow might, therefore, be considerable. However, Aston and Bradshaw (1966) have demonstrated genetic differentiation over shorter distances (10m) in populations of Agrostis stolonifera on sea cliff sites.

Comparison of the character means and correlations after one month's and six months' growth would suggest that phenotypic flexibility was becoming increasingly evident in the greenhouse habitat. For example, plants, which after one month were prostrate in habit, became gradually more erect, although never as erect as the plants grown from seed from an erect population. Also

the leaf characters of the different populations began to converge. This result was surprising, since Böcher (1943) endorsed Pilger's (1937) classification of P. lanceolata into 2 varieties and 7 sub-varieties based on growth habit and spike length, after growing the plants under greenhouse cultivation for many months. However, in the above experiment, space was limited in the greenhouse and the plants were grown in trays under conditions of high density: this might tend to favour an erect growth habit. That the plants were severely restricted in growth could be deduced by the fact that in 5 months in the greenhouse an average of only 0.9 new leaves per plant were produced and the plants had, on average, 8 leaves. (In the main investigation on micro-differentiation described later, the plants growing in individual pots in the greenhouse had an average of 23 leaves, most of these being produced within 9 months). The two prostrate populations both showed flexibility in growth habit, while remaining significantly different from each other. Populations 3, 4 and 5 remained erect. Population 5 did have shorter leaves on average than the other populations.

In conclusion, therefore, this experiment showed that when seeds from contrasting populations were grown to adults under uniform conditions, statistically significant differences were found between the populations with respect to the parameters studied. These differences correspond with those observed to exist between the parent populations and suggest that they are therefore the product of genetic variation.

2:2 Response of 'Prostrate' and 'Erect' Plantago Populations to Shading

Methods

An experiment was carried out to discover whether predominantly prostrate or erect plantain populations would differ not just in their morphology but also in their response to environmental change. Response to shading was studied as this seemed an obviously relevant environmental parameter.

Seed from population 1, mostly prostrate plants from exposed rocks near the sea, and population 4, mostly erect plants from an overgrown garden (see table 1, p 13) were used. For each population, 4 boxes containing 28 seeds spaced 5cm apart in John Innes No. 1 potting compost were set up in April 1970. In two of the boxes a sward was simulated by placing 9inch tall 'Twist-it' plant ties vertically between the seeds. The ties were made of wire sandwiched between paper $\frac{1}{4}$inch wide. The 8 boxes were then arranged in random order in an unlit greenhouse. Measurements of leaf number, leaf length, leaf width and growth habit were taken 5 months after sowing. None of the plants flowered in this time.

Results

The shade treatment had a significant effect on the leaf length and growth habit of the plants (tables 4 and 5 overleaf; Appendix 1, table 4, p 195), although there were still morphological differences between the prostrate and erect populations (as already noted from the previous experiment).

Table 4 Response to shading - morphology of prostrate and erect Plantago populations

Treatment	Population 1 - Prostrate				Population 4 - Erect			
	Leaf no.	Leaf length (mm)	Leaf width (mm)	Growth habit (1-5)	Leaf no.	Leaf length (mm)	Leaf width (mm)	Growth habit (1-5)
Unshaded	7.4 ±0.5	141.9 ±7.0	14.5 ±0.7	1.9 ±0.1	5.8 ±0.4	258.8 ±11.1	11.8 ±0.6	2.8 ±0.1
Shaded	6.9 ±0.5	228.2 ±8.1	14.9 ±0.6	2.6 ±0.9	6.3 ±0.5	249.3 ±12.0	13.6 ±0.9	3.1 ±0.1

Table 5 Response to shading - summary of analyses of variance on morphology

Source of variation	Character			
	Leaf no.	Leaf length	Leaf width	Growth habit
A Population i.e. growth habit	*	***	**	***
B Shade treatment		***		***
AB Interaction		***		***

(significance levels: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$)

The prostrate plantain population was more responsive than the erect population to the shade treatment and might therefore be the more flexible. This is shown by the significant effect of the shading on the leaf length and growth habit of the plants. They produced longer leaves and became very much more erect when shaded. The erect plants also responded to the shade treatment, tending to produce slightly shorter leaves and becoming more erect. The prostrate shaded plants, however, did not become as erect as the erect, unshaded plants nor did the leaves ever

become as long. Thus, although both populations of plants showed phenotypic flexibility, the extent of the differences between them was never wholly accounted for by this, indicating that a genetic component was present.

Discussion

The results of this experiment confirmed the result of the previous experiment, namely, that there were significant genetic differences between different populations of P. lanceolata. They also demonstrated differences in phenotypic flexibility. The more flexible prostrate population tended to grow taller when artificially shaded, but did not attain the height of the erect population, showing that there may be limits to the responsiveness of some populations.

The lower response of the erect population in this experiment is somewhat surprising. In the field, one would have supposed the erect plants to be exposed to greater seasonal variation in shading from surrounding plants and therefore to be more flexible. However, the plants were grown at high density in the greenhouse. In the unshaded situation, once the erect seedlings had grown a little, they may have shaded each other because of their erect habit whereas the prostrate seedlings may have shaded each other rather less. Consequently, the experimental difference between the shaded and unshaded plants may have been smaller for the erect than for the prostrate plants, giving the appearance of less flexibility. The greater flexibility of the prostrate population may, however, reflect the fact that prostrate plants tend to be found in open, exposed habitats. Flexibility would

be of selective advantage to prostrate plants, which would be more subject to changing climatic conditions than erect plants, buffered by the surrounding vegetation.

Both of the experiments show that P. lanceolata can undergo adaptive genetic differentiation in contrasting habitats. It is therefore a very suitable subject for research into the occurrence of adaptive genetic differentiation in a small heterogeneous environment.

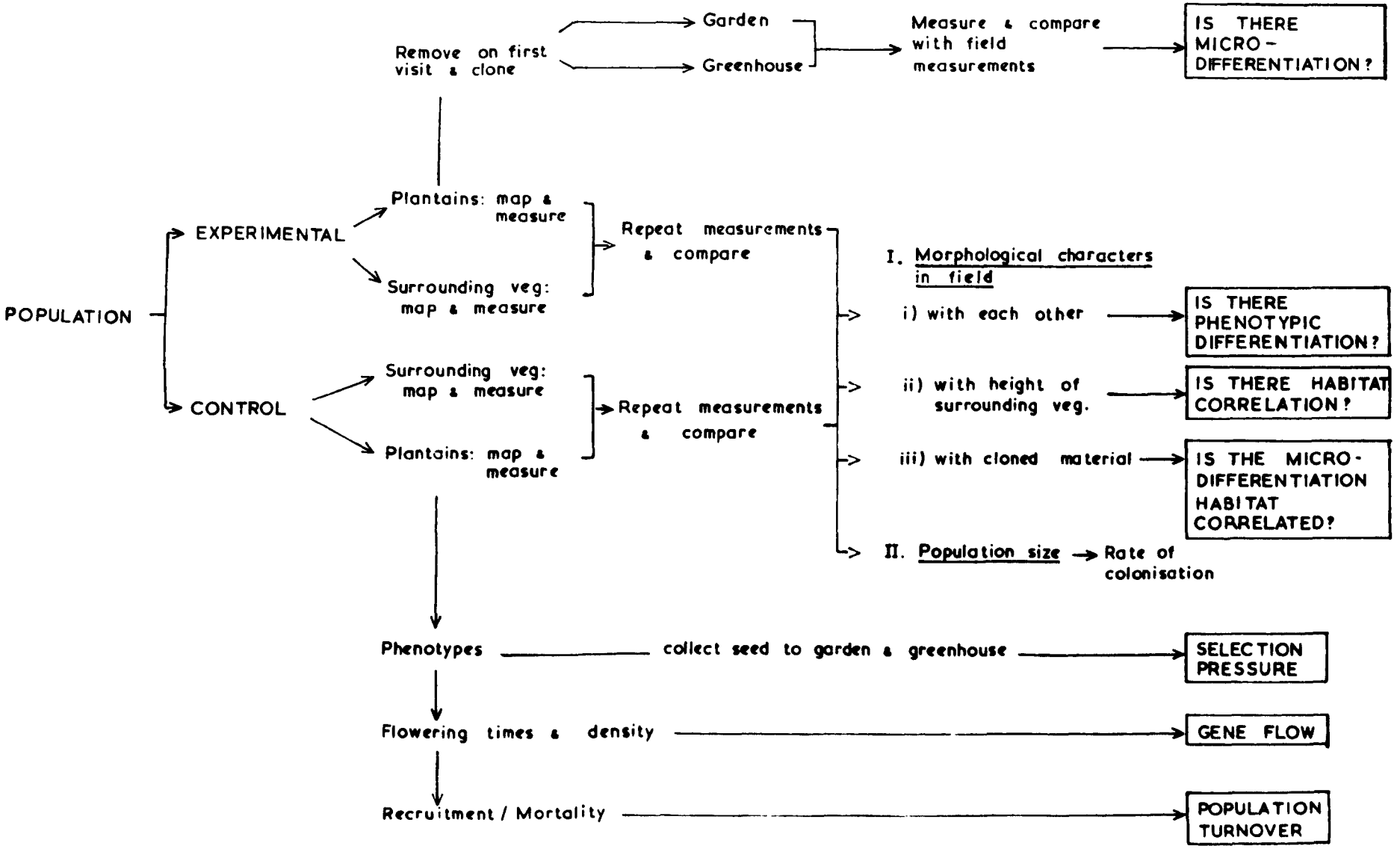


Fig. 2 Outline of field study.

3. Study of Micro-Differentiation in a Field Population of P. lanceolata

Methods

i) Experimental outline

An outline of the whole study is presented in fig. 2 as an annotated flow chart. A small field site containing a population of P. lanceolata of different phenotypes, was divided into two roughly comparable areas - an experimental strip and a control strip. The general strategy was to remove P. lanceolata rosettes from the experimental strip and grow them under uniform conditions. They were then to be compared with the micro-environment from which each plant was sampled in order to assess whether micro-differentiation was evident in the population. If adaptive differences were found between the plants in the field and these differences persisted after growth in uniform conditions, this would suggest micro-differentiation with a genetic component in the population. The micro-environment was defined and estimated in terms of vegetation height, vegetation composition, and in terms of the actual phenotypes of the experimental strip on sampling. The characteristics of the sampled plants were measured on cloned material in the two uniform, but different, environments of the gardens and greenhouse approximately one year after sampling. (Two uniform environments were used for purposes of statistical analysis). The control strip was used to study the phenology, population biology and flowering times, since these various parameters were considered important in the documentation of the evolutionary characteristics of the population. The

population and habitat in the control strip were more or less equivalent to that in the experimental strip.

The control strip and experimental strip were measured every three months; the sampling of the experimental strip was carried out on the first date. A detailed work schedule is given in Appendix 1, fig. 1, p 196.

ii) The population and its location

The population studied consisted of approximately 400 P. lanceolata rosettes, in a small area of 1 x 5m in dune pasture community at Lundin Links, Fife, Scotland (OS sheet 56, grid reference 405023). (See plate 1). The upper boundary of the site was adjacent to the fenced perimeter of the golf course which was constructed before 1939. Separating the dune from the sea shore was a breakwater, said by local residents to have been constructed immediately after the 1939-1945 war. It was therefore estimated that the dune pasture had been stabilised for at least 20 years. The small study area encompassed a graded patchwork of heterogeneity, variable in terms of vegetation height and species composition. A description of the general layout of the site, the ground profile, the position of the rosettes, the vegetation height and species composition, is given later (results i), p 38). The area, which traversed several micro-habitats, was divided into two 5m strips, each $\frac{1}{2}$m wide, which were very similar in general appearance. The plants in one strip, known as the experimental strip, were removed for cloning and study under experimental conditions while the other strip, known as the control strip, was left undisturbed for

Plate 1 General view of the study area at Lundin Links, Fife. (March 1970).

study of phenology and population dynamics. Each strip was divided into ten $\frac{1}{2}m^2$ quadrats.

iii) Cultivation and cloning

In June 1969 each plantain in the experimental strip at Lundin Links was removed,with as much rootstock as possible, to the greenhouse. The plants were grown for one month in John Innes No. 1 potting compost in 5in. pots in random order under normal greenhouse management. They were then cloned by making a transverse incision mid-way down the rootstock (see Appendix 1, p 197, for details of a cloning experiment). In late September 1969, two replicates of each plant were put out in the gardens. The plants were spaced 2ft apart and were in two blocks. Of the remaining plants in the greenhouse, some were recloned in April 1970 and others again in May 1970. Four replicates of each plant were then arranged in random blocks in the greenhouse. It was necessary to clone on more than one occasion to get sufficient replication because many plants were lost in the cloning process. It was thought that this might be due to the wide age range of the sampled plants - some cloning well and others not at all.

iv) Mapping technique

A graduated quadrat (called a plotter) was designed and constructed to map accurately individual rosettes within each $\frac{1}{2}m^2$ quadrat on the study site (plate 2, fig. 3). The framework of the plotter was of 'Speed Frame', with sections of metre rules glued to the upper surface of two sides. On this framework

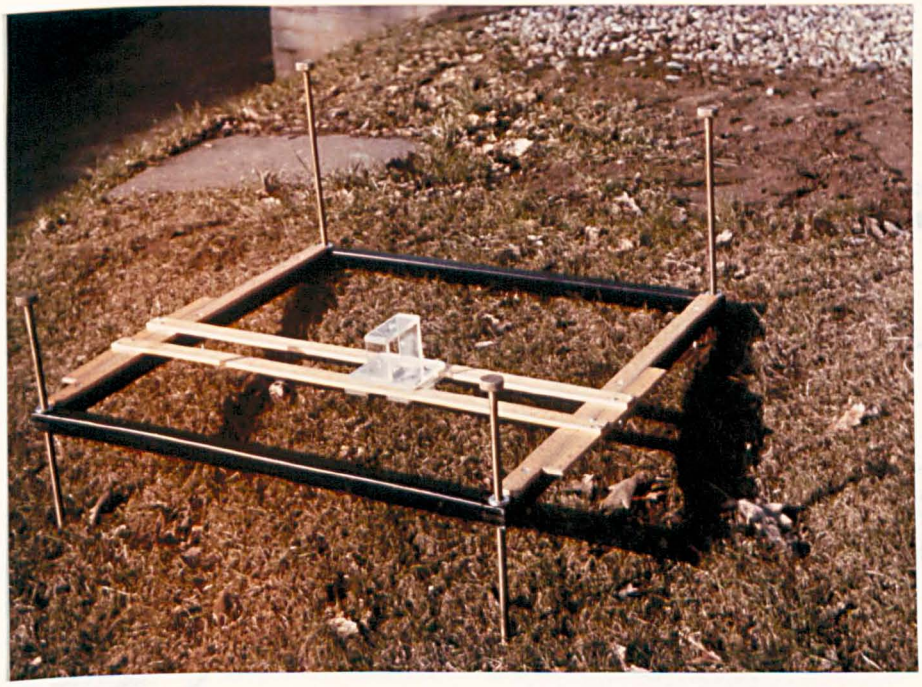


Plate 2 The 'plotter'.

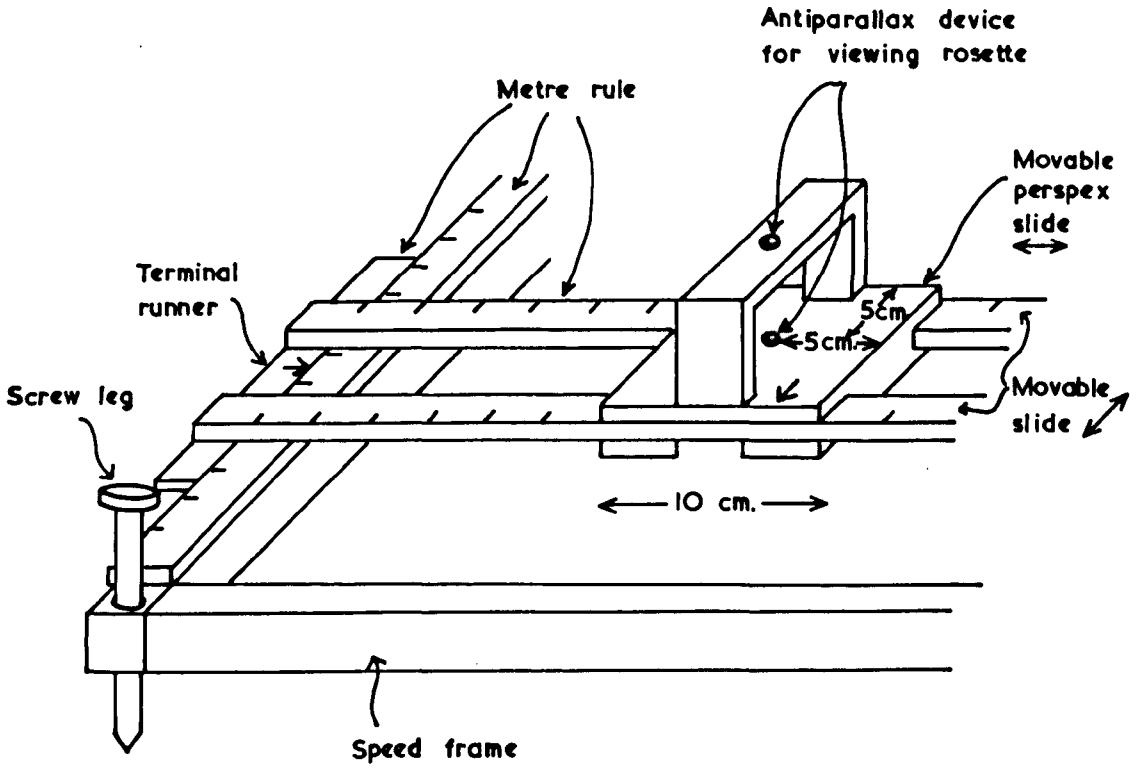


Fig. 3 Details of the 'plotter'.

rested a moveable slide made of two metre rule segments, joined by terminal runners. The moveable slide itself carried a perspex viewer designed as an anti-parallax device, through which the centre of a rosette could be viewed. Marks on the anti-parallax viewer enabled one to read off the y co-ordinate of the rosette on the metre rules constituting the moveable slide; marks on the moveable slide gave an x co-ordinate of the rosette when read from the metre rules glued to the quadrat frame. In this way each rosette could be mapped accurately and given an x and y co-ordinate to the nearest millimetre. The screw legs of the plotter rested on wooden pegs marking the position of the quadrats, and could be adjusted in height until the plotter was horizontal as judged by a spirit level placed on the frame. The position of each quadrat area was marked permanently by wooden pegs, 1 x 1 x 12in. driven down more or less flush with ground level. The pegs were positioned such that the mapped areas were contiguous with no overlap or gaps.

v) Height of vegetation

This was measured to the nearest 5mm at 10cm intervals throughout each quadrat by means of the apparatus shown in fig. 4. The plastic petri dish top was lowered gently until the vegetation prevented it from falling further. The height of the vegetation was read from the scale.

This rather unconventional method of measuring vegetation height was chosen because on a point basis vegetation height depended very much on the precise location of the scale: the scale might fall in a small open segment or next to a thin blade of grass. The method used essentially averaged these detailed

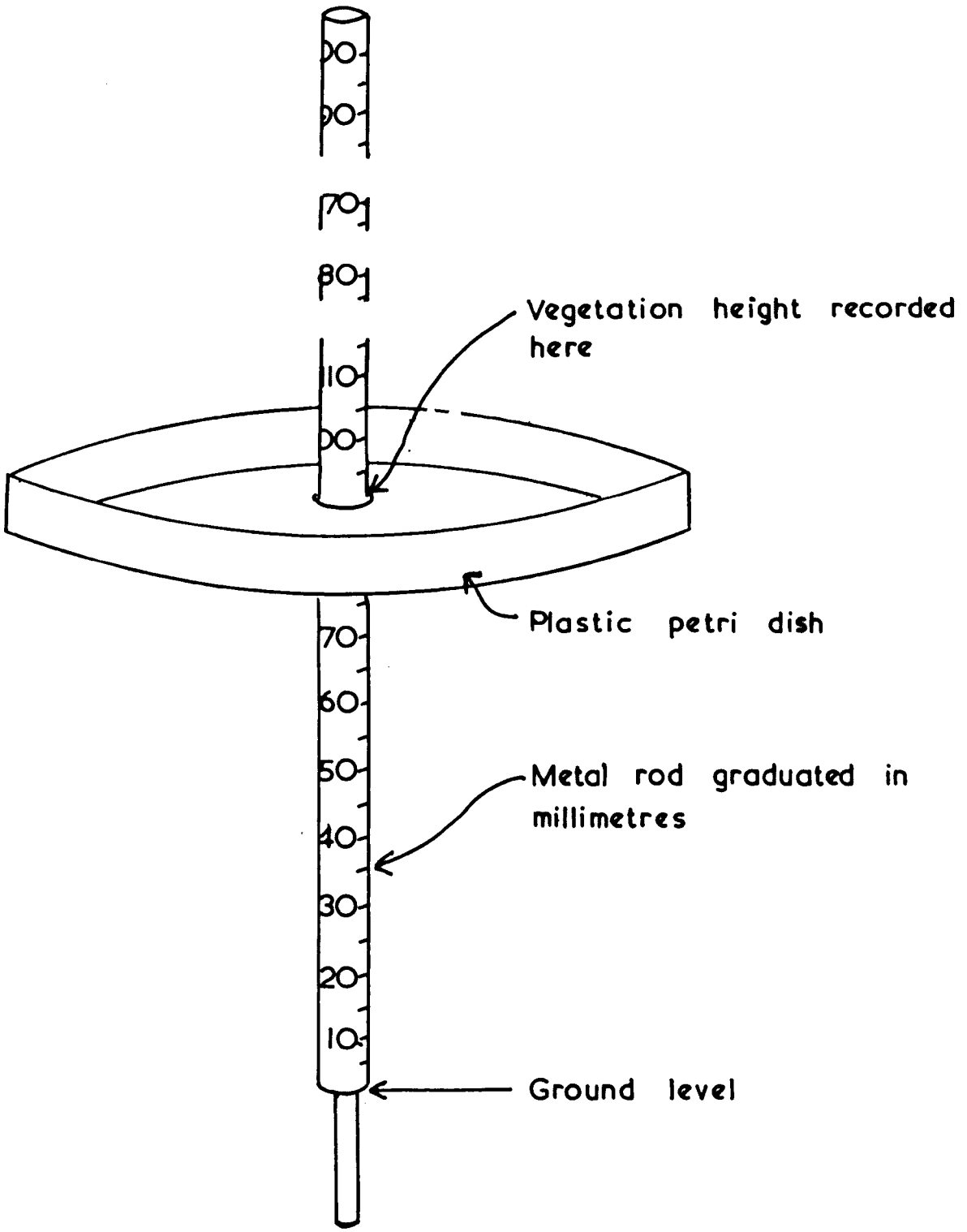


Fig. 4 Apparatus used to measure the vegetation height.

variations and gave a good reflection of general height of vegetation at each point.

From these readings contour maps were constructed with contours at every 25mm. Contour measurements were taken on the experimental and control strips every three months throughout the study. Overall means and standard deviations (variation in vegetation height) were calculated for every position, and contour maps constructed for mean vegetation height and variation in vegetation height (fig. 7, p 43).

Results

The results of the main investigation of *P. lanceolata* fall into three broad categories. The first of these (see i below) is a brief description of the area under investigation and the distribution of the *Plantago* rosettes within it. The second (results ii, p 50) deals with the investigation into micro-differentiation - the effect of the environment on the phenotypic and genotypic characteristics of the population, observed mainly from the experimental population sampled from the field and subsequently cultivated in the gardens and greenhouse. The third section (results iii, p 69) describes the population parameters, such as gene flow and population turnover, observed mainly from the control population in the field.

i) Description of the habitat and distribution of *P. lanceolata*

a) The habitat

A general layout of the area under investigation at Lundin Links is given in fig. 5, p 39. The control and experimental strips were approximately equivalent to each other and were patchy with respect to the density, type and height of the vegetation. A more detailed description is given below.

The profile of the ground was obtained by taking readings at 10cm intervals throughout each quadrat of the distance between the horizontal height of the plotter frame and soil level. Fig. 6, p 40, shows the average profiles obtained for the control and experimental strips. The site had a gradient of approximately

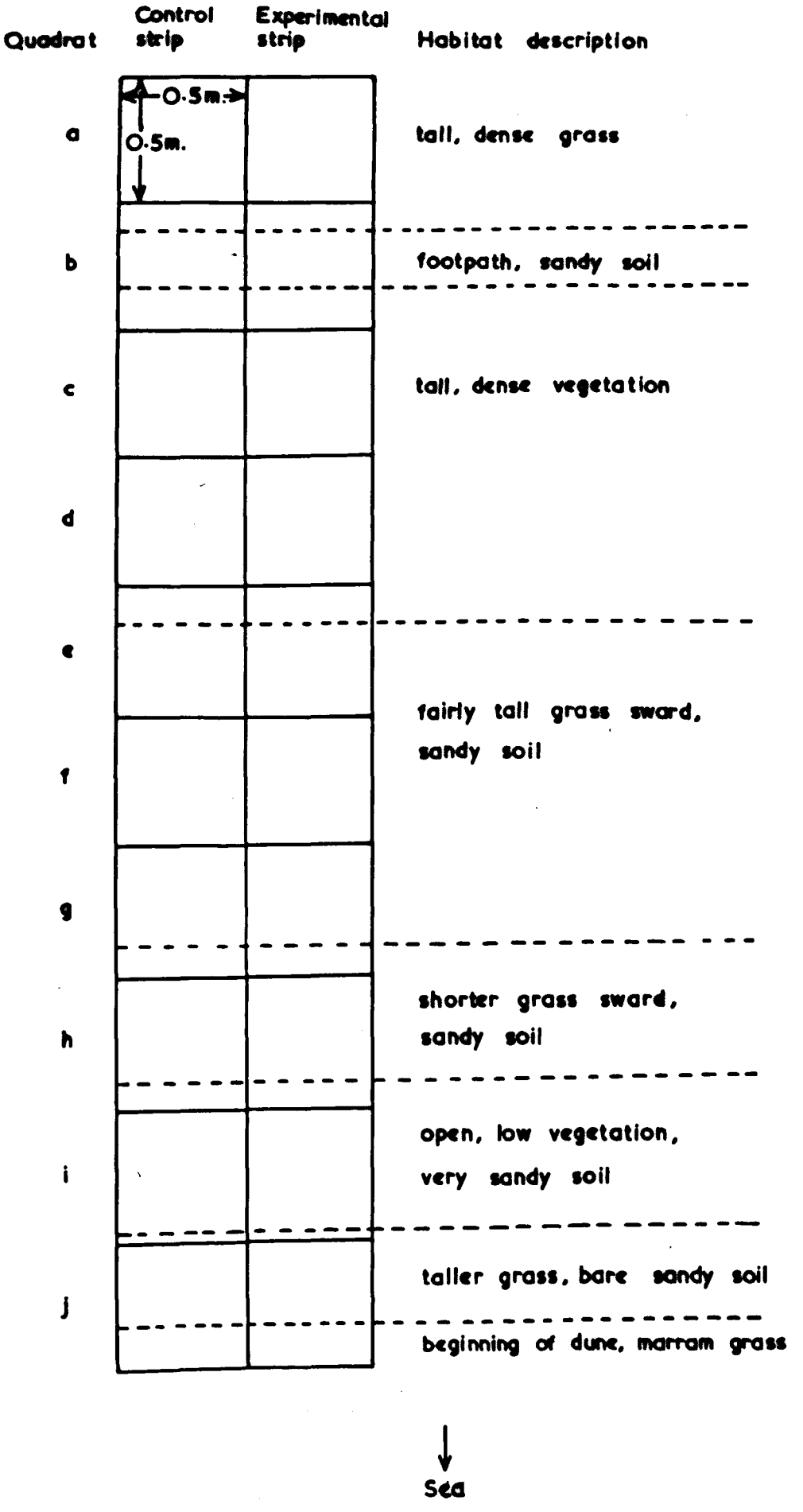


Fig. 5 To show layout of area investigated at Lundin Links.

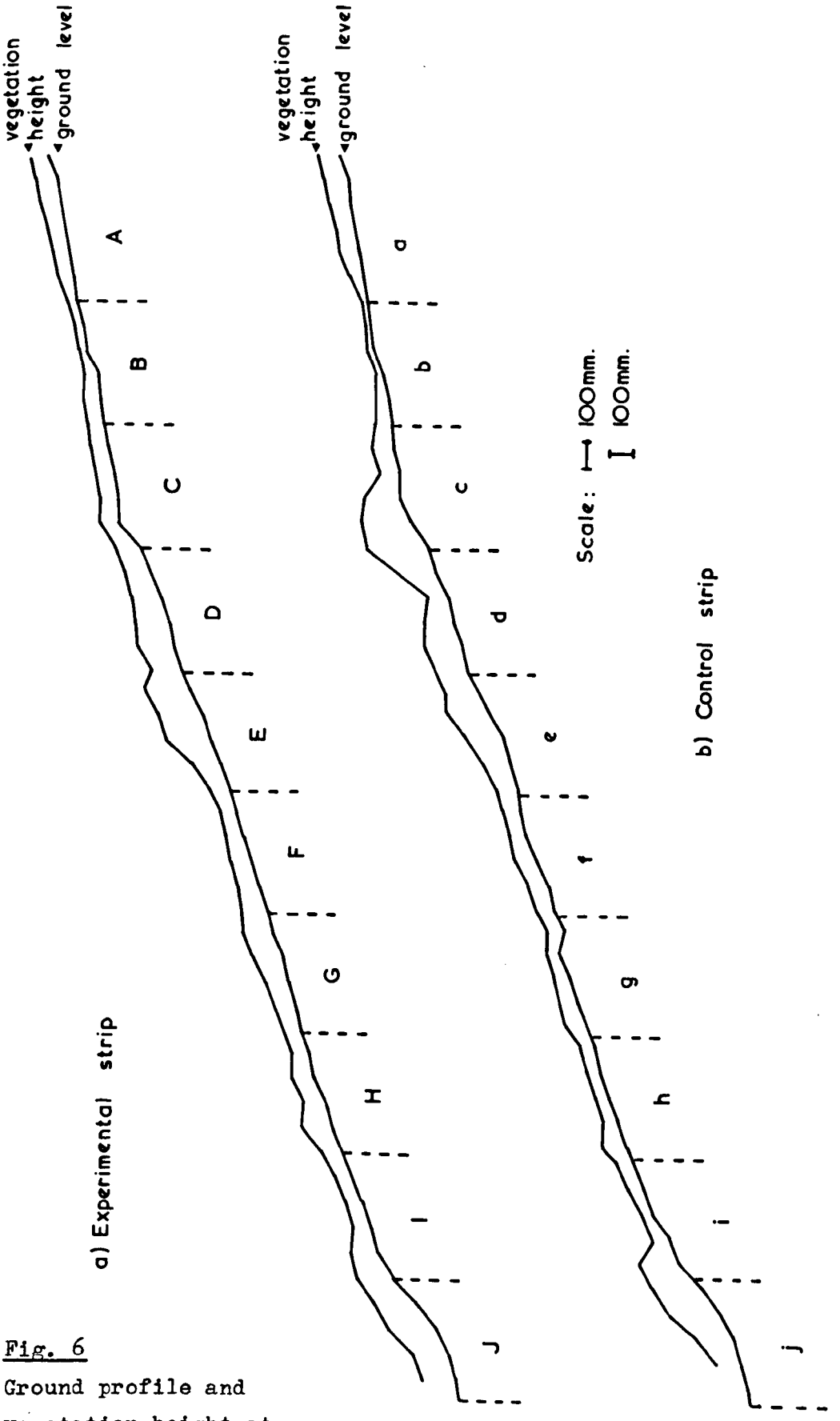


Fig. 6
Ground profile and vegetation height at Lundin Links in June 1969.

35% except in quadrats j (control strip) and J (experimental strip) where the dune pasture abruptly gave way to sand dune.

The contour maps (fig. 7, p 43) show the height of the vegetation in more detail. The lowest vegetation was in the region of the path (quadrats b, B) and on individual dates tall clumps of, for example, Ammophila arenaria were clearly detectable on the maps. The tallest vegetation showed the greatest variation (correlation coefficient = 0.66, $P \leq 0.001$) whereas the lowest vegetation on the path did not vary in height. The vegetation was of a more uniform height in December and March and showed the greatest height variation in June and September. The vegetation height did not vary greatly from year to year.

The vegetation in both the control and experimental strips was mapped fully in June 1970, using plotter scales. The species present were identified and their general areas marked. The results were collated as a vegetation map (fig. 8, p 45). Typical dune pasture species were present, Festuca rubra and Galium verum being particularly abundant. Ammophila arenaria was found in quadrats j and J where the dune pasture gave way to the sand dune.

b) Distribution of P. lanceolata

P. lanceolata was found throughout the area, although some quadrats were more densely populated than others (see fig. 9, p 46). Large numbers of rosettes were found in quadrats b, h, H, i, I and j. Very few were found in quadrats a and d. The rosettes on the control strip were shown to be non-randomly distributed by testing 'goodness of fit' to a Poisson distribution on a quadrat basis ($P \leq 0.001$). Table 6 shows the number of plants present in the

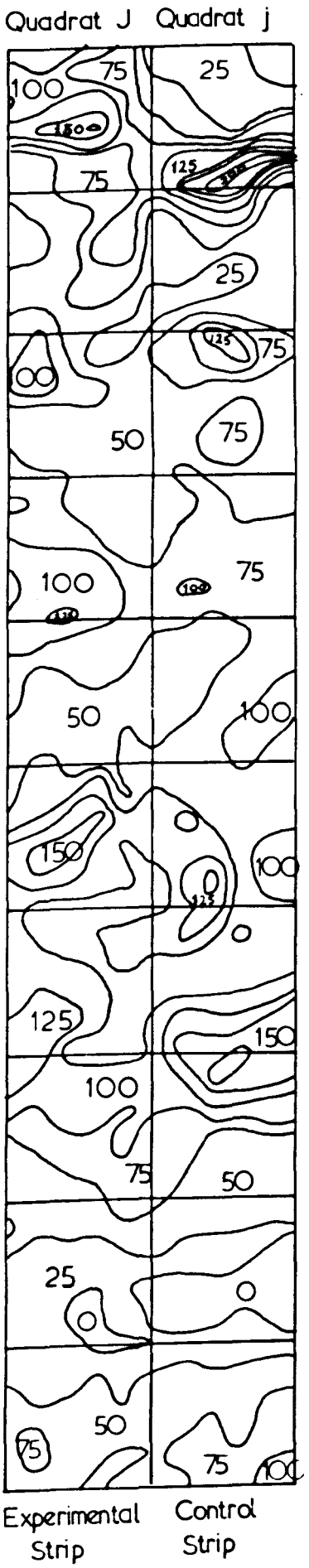
Figure 7.

Contour maps of the study area at Lundin Links

a) Mean vegetation height at 25mm intervals (June 1969 - September 1971)

b) Standard deviation of vegetation height at intervals of 10 units during the same period.

a)


Quadrat J
Quadrat j
Experimental Strip
Control Strip

b)

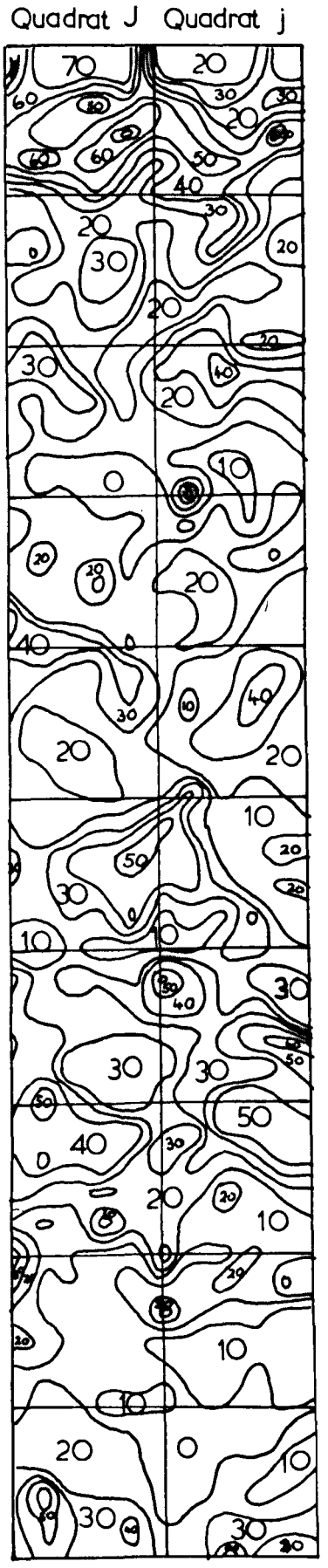

Quadrat J
Quadrat j

Figure 8.

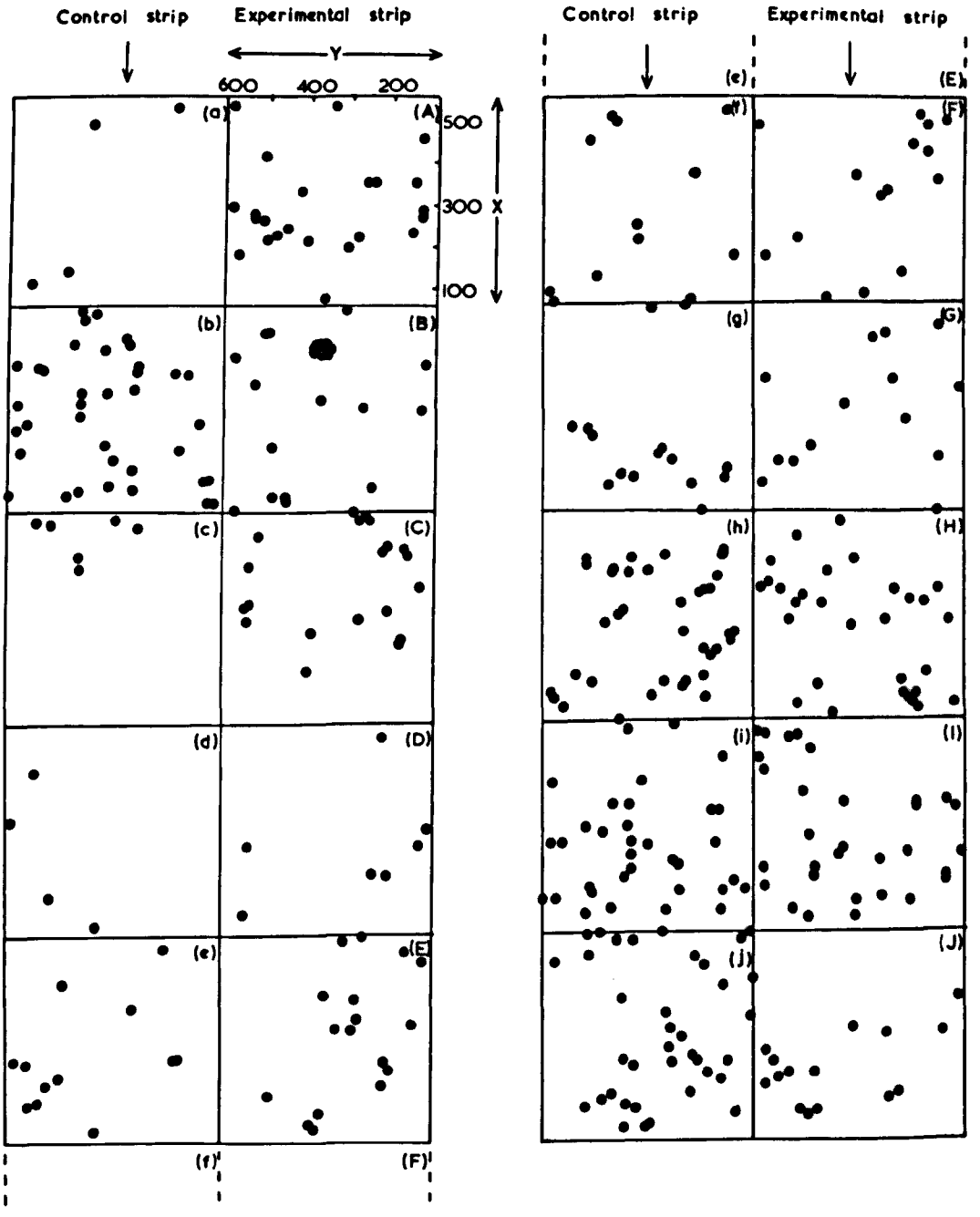
Examples of vegetation maps showing some of the more abundant species.

a) Achillea millefolium
Veronica chamedrys
Galium verum

b) Lotus corniculatus
Thalictrum minus
Ononis repens


a)
Quadrat J
Quadrat j
b)
J
j

Fig. 9 Position of mapped rosettes on the control and experimental strips prior to sampling in June 1969.



different quadrats on the control strip in June 1969, and also gives the distribution of the different phenotypes as classified by growth habit alone.

Table 6. Distribution of rosettes on the control strip in June 1969

Quadrat	No. plants	No. plants		
		P	I	U
a	4	0	2	2
b	38	27	10	1
c	6	0	5	1
d	4	0	2	2
e	11	0	5	6
f	12	0	6	6
g	14	1	7	6
h	36	0	19	17
i	36	12	22	2
j	34	0	8	26

Key:
P prostrate forms
I intermediate forms
U erect forms

Of the four most densely populated quadrats, b, h, i and j, quadrats b and i contained a high number of prostrate plants. From the contour maps (fig. 7, p 43) it can be seen that the lowest vegetation was found in quadrats b and i. The least densely populated quadrats, a, c and d, with tall vegetation, contained no prostrate plants.

One might speculate that the non-randomness of the population could be due to several causes, for example, vegetative propagation of individual plants, seed dispersal or environmental factors such as large areas of dense vegetation. Therefore in order to detect the approximate areas over which non-randomness (or clumping) pertained, a more detailed pattern analysis of the position of the

plants on the control and experimental strips in June 1969 was made using methods described by Greig-Smith (1964). In this technique, each quadrat is divided into a series of smaller but equal sub-divisions. The variance in the number of plants present in blocks of different size is then plotted against block size to detect at what levels clumping occurred (fig. 10, p 49). Because the variance increases as the block size increases, the data must be transformed by the regression of the plant position along the strip against the density of the plants. The deviation from the best line through these points is used to recalculate the plant densities (see Appendix 1, table 6). When treated in this way, the transformed data showed clumping of the plants at i) $\frac{1}{64}$ quadrat size = $62.5mm^2$, ii) $\frac{1}{8}$ quadrat size = an area of 125 x 250mm, iii) 4 x quadrat size = $1m^2$.

The first of the areas over which clumping was found ($62.5mm^2$) corresponds to a very small area around a plant and may be due to vegetative propagation. 4.3% of the plants were seen to be propagated in this way. This percentage may be artificially low as only those rosettes which shared a common rootstock were counted as clones. Clumping over an area of 125 x 250mm might be due to seed dispersal. The seeds of P. lanceolata tend to fall around the plant rather than to be dispersed over large distances as there is no active dehiscence mechanism (Sagar & Harper 1964). The third level of clumping (4 x quadrat size) is probably due to major environmental differences such as height or density of the surrounding vegetation. Many other causes, e.g. the level of other species, could be suggested.

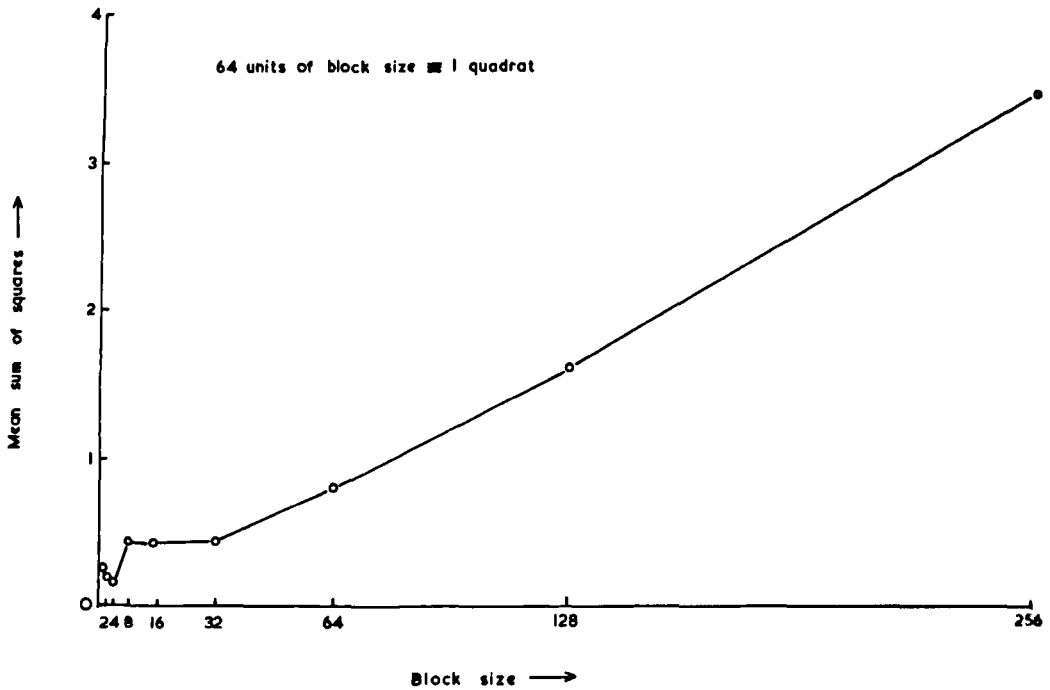


Fig. 10 Pattern analysis (after Greig-Smith 1964) to show levels of clumping of rosettes on the control and experimental strips in June 1969 (transformed data).

ii) The environment and its effects on phenotype and genotype - microdifferentiation

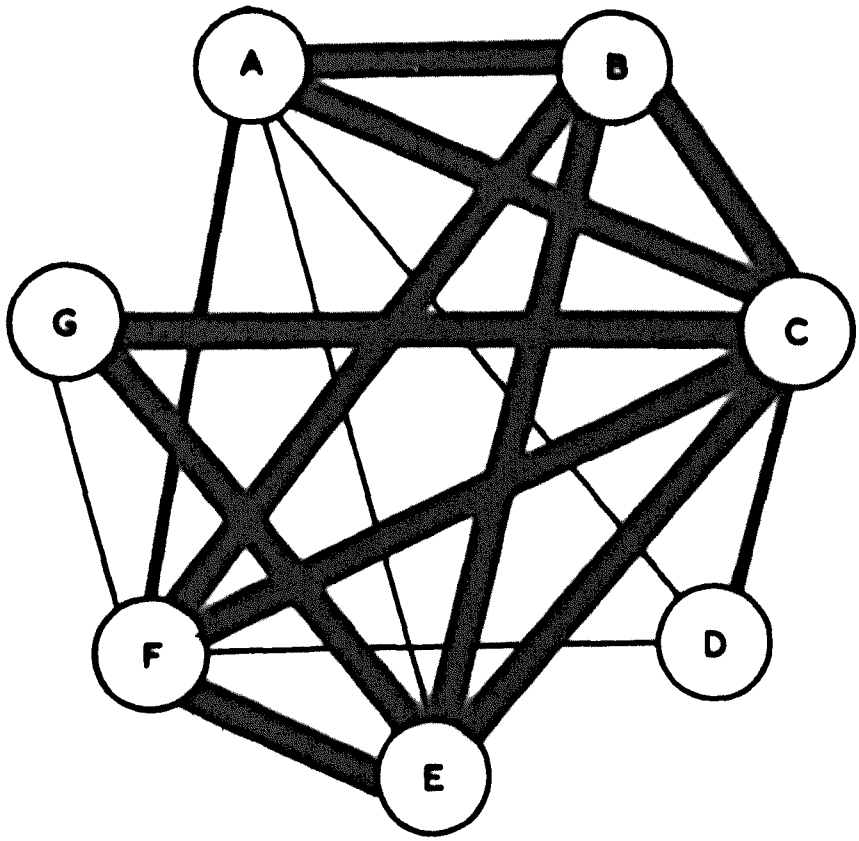
The experiments on contrasting populations (pp 14 - 28) showed the existence of different phenotypes of P. lanceolata. The first aim of the investigation on microdifferentiation was therefore to confirm the presence of these same phenotypes in the population at Lundin Links. This was achieved by correlating the morphological characteristics of the plants.

a) Morphology of the plants in the field

The individuals on the control strip at Lundin Links were measured for leaf number, leaf width (mm), leaf length (mm), inflorescence number, scape length (mm), spike length (mm) and growth habit. (This latter character was scored every three months on a 1 - 5 scale, 1 being the prostrate growth habit and 5 being the erect growth habit). The correlations between these phenotypic measurements taken in June 1969 are shown diagrammatically in fig. 11, p 51 (see also Appendix 1, table 7, p 200 , for correlation matrix).

From the diagram it can be seen that there is a high degree of significant positive correlation between the characters, e.g., leaf length is significantly positively correlated with all other characters. Inflorescence number and growth habit are the characters which are the least highly correlated with the other characters but they are both correlated with leaf length and spike length. It is therefore apparent that a gradation of phenotypes occurs - those at the one extreme having shorter, fewer leaves, shorter scapes and spikes and a prostrate growth habit, and those at the

Fig. 11 To show the significant correlations between the morphological characters of the plantains in the field in June 1969.



A	Leaf number	$p \leq 0.001$
B	Leaf width	$p \leq 0.01$
C	Leaf length	$p \leq 0.05$
D	Inflorescence number	
E	Scape length	
F	Spike length	
G	Growth habit	

other extreme having more leaves, longer leaves, longer scapes and longer spikes and an erect growth habit.

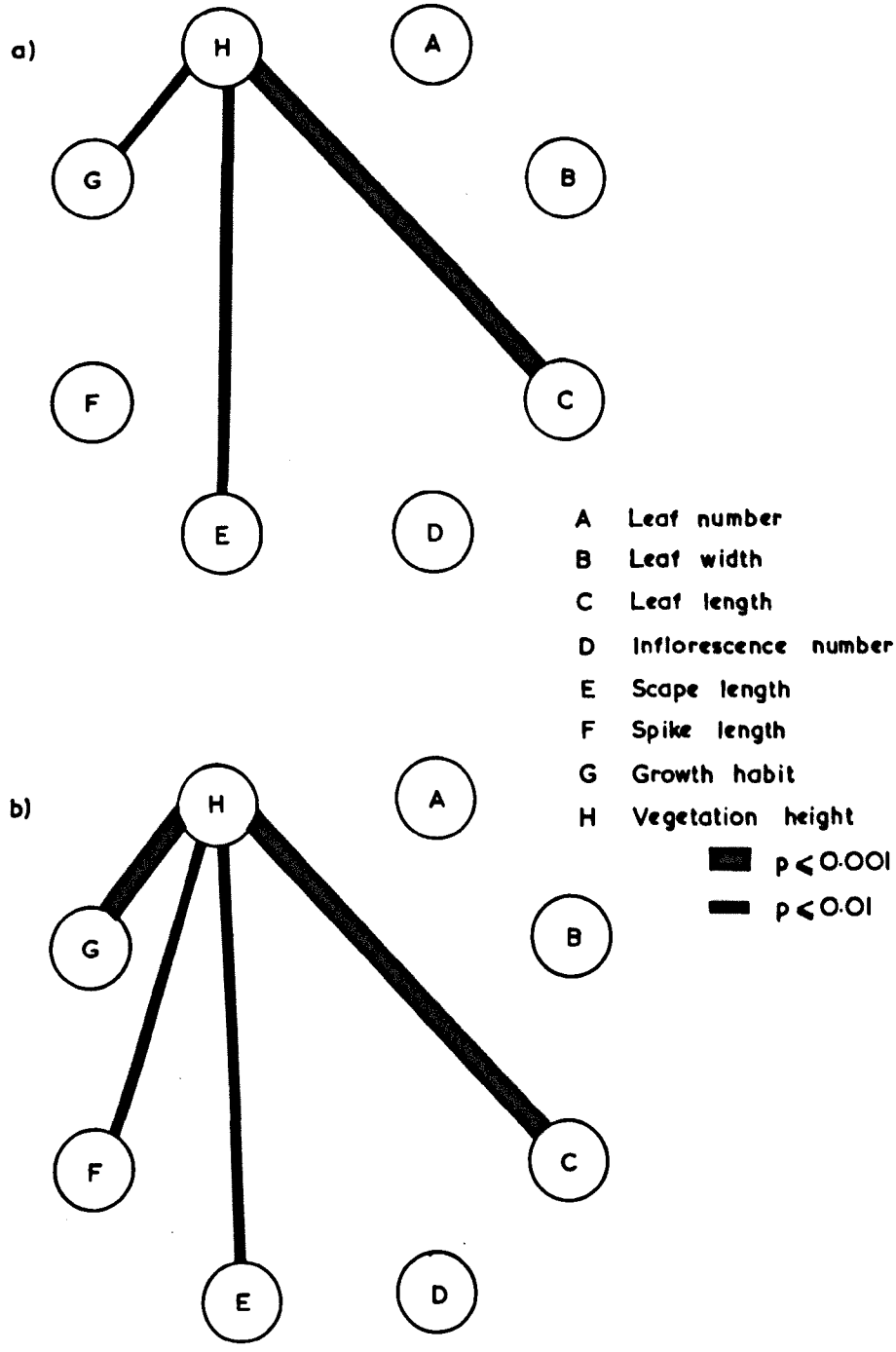
b) Morphology in relation to habitat, defined by vegetation height

The next objective of the investigation was to relate the field phenotypes to their habitat. The habitat was described in terms of vegetation height and individual plants were correlated with the height of the vegetation surrounding them.

The plantains, measured in the previous section (see p 50), were also mapped within each quadrat and given a grid reference. They were then replotted on the 25mm contour maps of June 1969 (fig. 7, p 43) and the height of the surrounding vegetation (to the nearest 25mm) was obtained for each individual. This was then correlated with the data collected in June 1969 for each plant (see results iia, p 50). The height of the surrounding vegetation was positively correlated with growth habit, leaf length and width, scape length and spike length ($P \leq 0.01$). Therefore, the taller the vegetation, the more erect and taller the plants tended to be. (See fig. 12a, p 53; also Appendix 1, table 8, p 200).

This analysis used only June 1969 data for the experimental plants surviving in the greenhouse. June 1969 data were used for this initial correlation because a) they reflected the state of the site before the experimental plantains were removed; b) the plantains which were removed may themselves have influenced the height of the vegetation; c) the site was relatively undamaged by trampling. In order to establish whether the correlations between the height of vegetation and leaf length, growth habit and

Fig. 12 To show the significant correlations between the vegetation height and the morphological characters of the plantains in the field a) in June 1969; b) in June 1970.



number of inflorescences were persistent, the individual plantains in the control strip in June 1970 were located on the 25mm contour map of mean vegetation height and measurements of leaf number, leaf length, leaf width, inflorescence number, scape length, spike length and growth habit were tested for correlation with mean vegetation height. It was found that leaf length, scape length and growth habit were highly positively correlated with mean vegetation height ($P<0.01$) as before. Leaf width was not correlated with mean vegetation height as in June 1969 but spike length was still correlated ($P<0.01$). (See fig. 12b, p 53; also Appendix 1, table 8).

There is therefore clear evidence that leaf length and growth habit of plantains in the field are generally correlated with vegetation height. These are probably the most important of the characters used for describing the phenotypes of the plants. It is apparent therefore that the phenotypes in the field are closely related to their habitats, as defined by vegetation height.

c) Genetic determination of phenotypic differences

The main strategy of the investigation on micro-differentiation was to find out, by statistical analysis, whether the phenotypic differences observed in the field (results iia, p 50) would persist after removal of the plants to two different uniform conditions, indicating genetic adaptation to their environment, i.e. micro-differentiation.

Therefore in June 1969 each plantain on the experimental strip at Lundin Links had been removed to the greenhouse and cloned. (See Appendix 1, p 197 and methods iii, p 32). Two replicates

had been set out in the gardens in late September 1969 and four replicates were kept in the greenhouse. Measurements of leaf number, leaf width, leaf length, inflorescence number, scape length and spike length were made in June and September 1970 on both garden and greenhouse plants. Growth habit was scored on eight occasions throughout the summer. This data, together with the field data (June 1969) of the corresponding plants, was then analysed by selective multiple regression using maximum R^2 improvement techniques, product-moment correlation and Spearman Rank correlation (see Appendix 1, p 201, for notes on statistical techniques). Spearman Rank correlation had to be used because growth habit is a discrete character. Analysis of variance was used to test whether there were differences between the genotypes of the plants within the garden and greenhouse environments.

Comparison of garden and greenhouse plants

When moved into the more favourable conditions of the gardens and greenhouse, the cloned plants showed a dramatic increase in size (table 7, p 56). The garden plants were much bigger than the greenhouse plants. Being spaced two feet apart in the garden and comparatively less restricted in growth than the potted plants in the greenhouse, they could support a larger root system. In order to get sufficient replication in the greenhouse, it had been necessary to clone the greenhouse plants more than once. There are interesting differences between the plants cloned at different times. Being younger, those of clone date 2 have fewer leaves and fewer inflorescences than those of clone date 1. However, the leaf dimensions and inflorescence dimensions are also different. The

Table 7. Comparison of character means of plants in the field with means obtained after cloning and cultivation in garden and greenhouse environments

Character	Means			
	Field 1969	Garden	Greenhouse 1	Greenhouse 2
Leaf no.	3.9 ±0.2	42.4 ±2.8	23.6 ±1.1	10.2 ±0.4
Leaf width (mm)	9.4 ±0.3	22.9 ±0.6	11.8 ±0.2	14.9 ±0.3
Leaf length (mm)	126.7 ±5.4	320.9 ±8.2	149.8 ±3.7	184.6 ±3.9
Inflorescence no.	1.8 ±0.2	50.6 ±2.5	12.7 ±0.5	4.3 ±0.3
Scape length (mm)	211.6 ±13.0	546.9 ±9.6	315.0 ±5.8	379.1 ±7.9
Spike length (mm)	10.1 ±0.6	47.9 ±1.4	3.3 ±0.6	21.7 ±0.6
Growth habit (1-5)	4.0 ±0.1	3.7 ±0.1	3.3 ±0.1	3.8 ±0.1

Note: Greenhouse 1 - Clone date October 1969
Greenhouse 2 - Clone date April 1970

younger plants have larger leaves and larger inflorescences. These plants were cloned shortly before the flowering season and it seems that the plants' energy may have been redirected into producing fewer, larger leaves and inflorescences.

Analysis of variance on garden and greenhouse plants

It was crucial to the investigation to find out whether the phenotypic differences observed in the field still persisted in the two different, though uniform, environments of garden and greenhouse in spite of the overall increase in size of the plants. Therefore 1) a one-way analysis of variance was carried out to determine whether there were detectable genotypic differences regardless of environment and 2) a two-way analysis of variance was carried out in order that the variance might be partitioned

to obtain heritability data. However, because of clonal death, only 39 of the 119 sampled plants were fully replicated in both garden and greenhouse and were available for this analysis. Since, therefore, the analysis was based on a small sample, the error would be expected to be high. Although the significant results obtained would be valid, other real genotypic differences might not be apparent. Gross heritability for growth habit could not be calculated as it was not possible to do a two-way analysis of variance on the qualitative data obtained for that character.

The results of the one-way analysis of variance are summarised in table 8, which shows the significance levels where differences between genotypes were found.

Table 8. Significance levels obtained from one-way analysis of variance between genotypes within environments for the morphological characters of the cloned plants

Character	Garden		Greenhouse			
	June	September	June		September	
			Clone 1	Clone 2	Clone 1	Clone 2
Leaf no.			***	***	***	***
Leaf width		***	***	***	***	**
Leaf length	*	***	***	***	***	***
Inflo. no.			**		***	
Scape length	*	***	**	**	***	***
Spike length		***	***	**	***	**

Key: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$

Strong genotype differences (as compared with replicate differences) were found for leaf length and scape length, regardless

of the cloning date or in which environment (garden or greenhouse) the plants were situated. (It will later be found that these characters are always highly correlated). Leaf length and spike length showed strong genotypic differences in both gardens and greenhouse but leaf number and inflorescence number only showed genotypic differences in the greenhouse.

To quantify the probable genetic component determining these characters (except perhaps leaf number and inflorescence number), a calculation of the gross heritability for main effects was made by partitioning the variance obtained from the two-way analysis of variance. Gross heritability was determined from the formulation:

Gross heritability for main effects (h^2) = degree of genetic determination

$$= \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e}$$

where $\sigma^2 g$ = genotypic variance and $\sigma^2 e$ = pooled environmental variance and environmental variance between replicates.

From the analysis of variance table, the variance may be partitioned as follows:-

Variation	Mean Sum Sq Components
Genotypes	$r\sigma^2 g + \sigma^2 e$
Error	$\sigma^2 e$

From Huitson (1966), r is approximately 3, therefore h^2 may be calculated.

Table 9 shows the heritabilities of the different characters in the different environments calculated as above:

Table 9. Heritabilities based on 2-way analysis of variance

(June and September 1970 data)

	Gardens		Greenhouse			
	June '70	Sept '70	June 1970		September 1970	
			Clone 1	Clone 2	Clone 1	Clone 2
Leaf no.	-0.01 (NS)	-0.29 (NS)	0.58	0.53	0.51	0.56
Leaf width	-0.13 (NS)	0.73	0.50	0.26	0.37	0.20
Leaf length	0.47	0.78	0.32	0.29	0.39	0.51
Inflo. no.	0.30 (NS)	0.38 (NS)	0.28	-0.02(NS)	0.38	0.08(NS)
Scape length	0.43	0.75	0.29	0.53	0.45	0.44
Spike length	0.33 (NS)	0.64	0.33	0.53	0.59	0.29

All heritabilities significant at 5% level except those marked (NS)

The negative heritabilities are probably explained by the large error due to the small numbers involved. It is clear, since so many of the heritabilities are significant, that the characters, except perhaps inflorescence number, are genetically determined to a major extent, especially leaf length and scape length, in which all the heritabilities are significant and of high value in the gardens. Because the calculation of gross heritabilities represented one of the aims of this investigation, it was included even though little weight can be attached to the actual heritabilities calculated. A more efficient and successful method of cloning P. lanceolata has since been devised by Lion Wu (pers. comm.) and if this method had been available there is little doubt that the heritabilities obtained would have been more accurate and, possibly, more significant.

Correlation analysis of garden and greenhouse plants

It was decided to confirm the results obtained by the analysis of variance above using an alternative method of analysis, which took into account all the cloned plants, including those not completely replicated in both the gardens and the greenhouse. Selective multiple regression using maximum R^2 improvement techniques, product-moment correlation and Spearman Rank correlation was used. The results are described in the following three sub-sections but for correlation matrices and notes on statistical techniques see Appendix 1, pp 200 - 207. It will be found that leaf length and scape length, together with growth habit, are genetically determined to a major extent.

1) Correlation of the plant characters after cultivation in gardens and greenhouse

The morphological characters of the plants in each of the uniform environments (garden and greenhouse) were correlated together to establish whether the phenotypic differences found in the original sample of plants had persisted despite the increase in plant size (see table 7, p 56). The highly significant correlations previously found tend to persist, e.g. leaf length, scape length and growth habit are still positively correlated. The weaker correlations found in the field (fig. 11, p 51) have now disappeared, i.e. those characters correlated with leaf number and inflorescence number. Fig. 13, p 62, shows the significant correlations between characters in the garden and greenhouse plants; fig. 13g isolates the most important and consistent correlations found in the different environments.

Figure 13

To show the significant correlations between the plant characters in the garden and greenhouse

a) Garden, June 1970

b) Greenhouse (clone date 1), June 1970

c) Greenhouse (clone date 2), June 1970

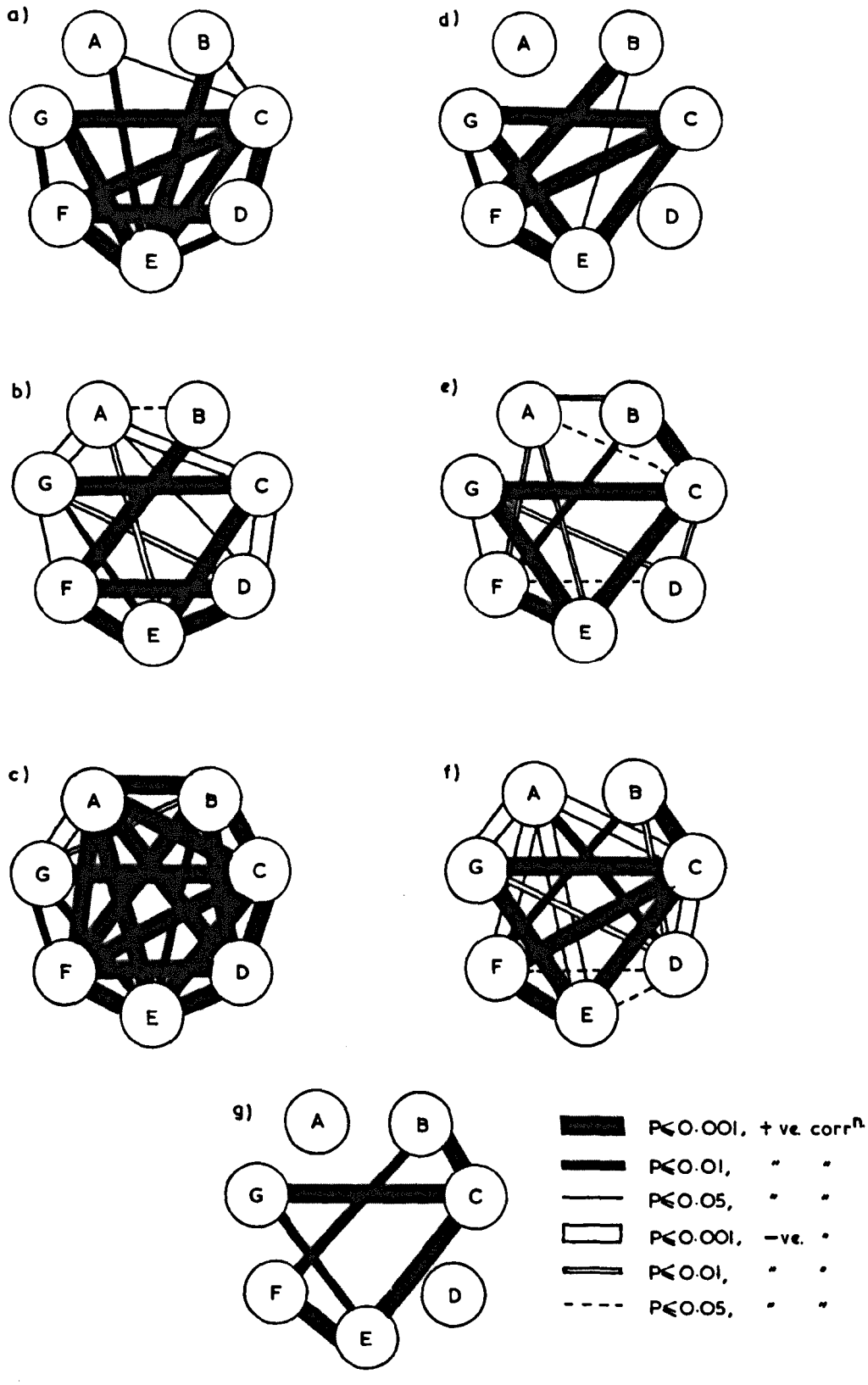
d) Garden, September 1970

e) Greenhouse (clone date 1), September 1970

f) Greenhouse (clone date 2), September 1970

g) the consistent and important correlations shown in a - f. (See appendix 1, table 11)

Key: A = leaf number; B = leaf width;
C = leaf length; D = inflorescence number;
E = scape length; F = spike length;
G = growth habit.


a)
b)
c)
d)
e)
f)
g)
A
B
C
D
E
F
G
P≤0·001, + ve. corrn.
P≤0·01, " "
P≤0·05, " "
P≤0·001, −ve. "
P≤0·01, " "
P≤0·05, " "

Therefore there is evidence that the phenotypic correlations found in the field persist when the plants are grown in uniform conditions in dissimilar environments in spite of large increases in general plant size. It is interesting that the correlations between leaf number and inflorescence number with the other characters are not consistently found, either in the field or in the different uniform environments. Where correlations with these characters do exist in the garden and greenhouse, they are found to be positive in some cases and negative in others, indicating that leaf number and inflorescence number respond to the environment independently of many of the other characters.

2) Correlation of garden and greenhouse phenotypes with vegetation height from which the plantains originated

It was reported earlier (p 52 and fig. 12, p 53) that the phenotypes in the field were closely related to the height of the vegetation surrounding them, plants from taller vegetation having longer leaves, scapes and spikes and being more erect in growth habit. It was now found by correlation of the vegetation height with the morphological characters (Appendix 1, tables 12 and 13, pp 204 - 205) that, even after cultivation in the garden and greenhouse, plants originally from taller vegetation have more leaves, longer scapes and spikes and are more erect in growth habit. Therefore three of the four correlations found earlier (p 52) have persisted under cultivation despite the large increase in size of the plants. This would indicate an underlying genetic component for these characters. An additional correlation (height with leaf length) is found in the field but this does not persist

with any level of consistency under cultivation.

The clearest relationship is between leaf number and vegetation height, but this significant correlation found in the garden and greenhouse, does not occur in the field. This correlation is interesting because the plants in the greenhouse and garden were growing in comparative isolation and were free to increase in size as much as the soil resources and space permitted: the mean leaf number of the greenhouse plants was 23 for those of clone date 1, 10 for those of clone date 2 and 42 for the garden plants, compared with 4 in the field, reflecting the influence that the environment had on the ultimate plant size. That these leaf numbers were correlated with the mean vegetation height in the field is somewhat surprising and reveals that the different phenotypes may be under differential limiting pressures in the field which may tend to mask the expression of genetic differences, e.g. erect plantains growing in tall vegetation may tend to be more shaded than prostrate plants growing in low vegetation, thus comparatively restricting the development of the erect plants and slowing down their growth rate. Alternatively it may indicate that in tall vegetation there is selection for rapid growth rate, but that environmental effects and the wide range of ages of plants in the field mask the correlation.

3) Comparison of plantains in the field with the same plantains grown in the garden and greenhouse

The characters of the plants in the field reflect both the direct influence of the environment (and are therefore in some measure a biological assay of the environment) and they reflect the genotype of the plants. If the genotypes are themselves associated with certain environments, then the field characters of the plants

Table 10 Correlation matrix of Phenotypes in field with phenotypes under standard conditions

Lumdin Links	Standard Conditions: Leaf No June	Leaf No Sept.	Leaf width June	Leaf width Sept	Leaf length June	Leaf length Sept	Inflo. No. June	Inflo. No. Sept	Scape length June	Scape length Sept	Spike length June	Spike length Sept	Growth habit June	Growth habit Sept	
Leaf No	0.10	-0.21	-0.01	0.08	-0.11	-0.02	-0.14	-0.13	-0.12	-0.05	0.03	0.06	-0.02	-0.16	A
	0.25	0.17	-0.11	-0.18	-0.06	0.07	0.02	-0.07	-0.00	0.03	0.05	-0.03	0.06	0.04	B
	-0.04	-0.07	-0.12	-0.07	-0.09	-0.14	-0.18	-0.09	-0.11	-0.15	0.10	0.04	0.03	0.05	C
Leaf width	0.05	-0.15	0.02	0.09	0.09	0.05	-0.16	-0.20	-0.06	-0.05	-0.08	-0.08	-0.02	0.04	
	0.16	0.03	0.01	-0.02	-0.05	0.11	-0.09	-0.12	-0.09	0.04	0.07	0.06	0.17	0.17	
	-0.09	-0.10	-0.16	-0.03	-0.16	0.06	-0.17	-0.12	-0.20	-0.01	-0.02	0.04	0.12	0.10	
Leaf length	0.09	-0.04	-0.07	0.24	0.26	0.27	0.02	-0.07	-0.01	0.11	0.01	0.12	0.37	0.32	
	-0.08	-0.11	0.04	0.05	0.05	0.18	-0.21	-0.26	-0.04	0.03	-0.02	-0.04	0.16	0.22	
	0.11	0.03	0.03	-0.05	0.03	0.22	0.08	0.00	0.07	0.16	-0.09	0.09	0.17	0.26	
Inflo No.	0.12	-0.27	-0.07	0.44	-0.18	-0.20	-0.21	0.04	-0.11	-0.09	0.04	0.26	-0.17	-0.21	
	0.32	0.23	0.15	-0.13	-0.01	-0.11	0.19	0.05	-0.13	-0.16	0.21	0.11	-0.32	-0.22	
	-0.07	0.12	-0.10	-0.00	-0.20	-0.34	-0.03	0.04	0.11	-0.39	0.44	-0.02	-0.06	-0.05	
Scape Length	0.23	-0.17	-0.19	0.20	0.32	0.28	-0.04	-0.08	0.06	0.17	-0.05	0.06	0.53	0.56	
	-0.04	-0.09	0.32	0.17	0.20	0.19	-0.35	-0.28	0.01	0.05	0.13	0.18	0.20	0.28	
	-0.22	-0.31	-0.19	0.03	0.08	0.48	-0.18	-0.13	-0.16	0.20	-0.26	-0.00	0.43	0.52	
Spike Length	0.16	-0.32	-0.19	0.42	0.15	0.16	-0.08	-0.06	-0.00	0.05	0.03	0.17	0.31	0.29	
	0.30	0.19	0.10	-0.15	0.15	0.19	-0.11	-0.06	0.04	0.08	0.29	0.34	0.09	0.07	
	-0.18	-0.24	-0.15	0.05	0.11	0.30	-0.10	0.01	-0.06	-0.12	-0.05	0.11	0.30	0.35	
Growth habit	0.03	0.07	0.01	0.07	0.18	0.23	0.09	0.05	-0.05	0.02	0.08	0.05	0.40	0.37	
	0.02	-0.05	0.03	0.05	0.09	-0.12	-0.04	-0.02	-0.07	-0.09	0.05	-0.05	0.11	0.05	
	-0.02	-0.05	0.03	0.05	0.14	0.23	0.01	0.02	-0.05	-0.01	-0.05	0.01	0.13	0.26	
Mean vegetation height	0.13	-0.14	-0.14	0.13	0.16	0.27	0.06	-0.04	-0.06	0.11	-0.07	-0.26	0.16	0.19	
	0.16	0.23	-0.01	0.07	0.01	0.12	-0.23	-0.20	-0.14	-0.08	-0.13	-0.10	0.05	0.12	
	0.20	-0.09	0.10	-0.14	0.08	0.15	-0.08	-0.02	-0.13	0.08	0.07	0.03	0.04	0.26	
Variance in vegetation height	0.21	-0.07	-0.06	0.26	0.15	0.23	0.10	0.03	-0.07	0.01	0.01	0.07	0.01	0.03	
	0.11	0.20	-0.09	0.03	0.00	0.14	-0.26	-0.26	-0.10	-0.04	-0.17	-0.14	-0.09	0.05	
	0.32	0.10	0.06	-0.24	-0.00	0.03	0.09	0.02	-0.16	-0.04	0.05	-0.03	-0.05	0.14	

A Gardens
B Greenhouse 1
C Greenhouse 2

Statistical significance of correlation coefficients:

If correlation coefficient ≥ 0.32, $P < 0.001$

If correlation coefficient ≥ 0.25, $P < 0.01$

If correlation coefficient ≥ 0.20, $P < 0.05$

confound three influences, namely, environment, genotype and microadaptation (genotype-environment correlation). The relationship between the phenotypes in the field and the phenotypes in uniform conditions is a measure of two things: a) whether the phenotypic differences observed in the field have in part a genetic component, and b) whether there is microdifferentiation in response to a heterogeneous environment, if the field characters are themselves correlated with the environmental differences. The only condition under which the latter would not hold is if the plants that were responsive to the environment were not the same as the plants which showed genetic differences. This seems unlikely.

Two categories of correlation were distinguished in the analysis:

i) Field characters correlated with the same characters under uniform conditions,

ii) Field characters correlated with other characters under uniform conditions.

Table 10 shows the correlation matrix, which combines both of these two categories of correlation. For full details see Appendix 1, tables 14 and 15. Leaf number, leaf length, spike length and the growth habit of the plantains when in the field are significantly correlated with those same characters of the same plantains when in the garden or greenhouse. Of these characters, leaf length is the most highly correlated and the correlations (leaf length in the field with leaf length in the garden and greenhouse) are positive. For leaf number, however, the correlation is negative in the garden but positive in the greenhouse, indicating the different effects the two environments have on that character.

The other consistent correlations of each character in the field with other characters under uniform conditions involve growth habit. Leaf length, scape length and spike length (as well as growth habit) in the field are all correlated with growth habit in uniform conditions. Growth habit in the field is in turn correlated with leaf length under uniform conditions.

This pattern of correlations strongly suggests that the characters of leaf length, inflorescence length and growth habit, which are correlated in the field, (see p 50) are genetically determined since these characters are also correlated under uniform conditions of cultivation.

d) Discussion

From the foregoing results, a picture emerges of a population of plantains incorporating the extremes of prostrate plants with smaller leaves and inflorescences living in low vegetation with the more erect plants with larger leaves and inflorescences living in taller vegetation, together with a gradation of intermediate types. These genotypes remain distinct when the plants are moved into different, more uniform environments, even though all the plants alter in size, especially increasing the numbers of their leaves and inflorescences. These characters (leaf number and inflorescence number) are highly influenced by the environment. The prostrate plants tend to grow more erect and the leaves and inflorescences become larger but, even so, they remain relatively smaller than the erect plants which tend to remain erect but also increase leaf and inflorescence dimensions. The characters of leaf length, scape length and growth habit are always highly correlated and one might suspect genetic linkage or developmental correlation between them.

Calculations of gross heritability for the main effects in the different environments of field, garden and greenhouse, confirm that there may be a fairly high degree of genetic determination for these characters, though a reliable quantitative assessment could not be made due to the small number of plants which were fully replicated in both garden and greenhouse. This analysis does assume, however, that after one year's growth in two different, but supposedly uniform, environments any remaining variation is genetic. Although this is likely, it might not be so since there is the possibility of somatic selection inducing semi-permanent changes in the phenotype (Durrant 1962).

iii) Population Parameters

The population of P. lanceolata in the control strip at Lundin Links afforded the opportunity to study various parameters, which were considered to be relevant to the investigation and the understanding of the dynamic forces acting on the population. These parameters consisted of one aspect of gene flow, i.e. reproductive isolation, selection pressure and population turnover.

a) Reproductive isolation

An investigation was carried out to determine whether there was reproductive isolation of any kind between the phenotypes. The divergent phenotypes in the heterogeneous habitat were situated only a few centimetres apart and it might be supposed that there would be heavy gene flow tending to swamp this divergence. The following parameters were measured for the prostrate, intermediate and erect phenotypes:- flowering times, number of inflorescences and the amount of seed set.

Flowering times

Flowering was arbitrarily divided into six stages:

1) appearance of bud
2) elongation of scape
3) appearance of stigmas
4) anthesis in lower half of spike
5) anthesis in upper half of spike
6) anthesis ended.

These stages are illustrated in plate 3.

Plate 3

The different flowering stages:

1) Appearance of bud,
2) elongation of scape,
3) appearance of stigmas,
4) anthesis in lower half of spike,
5) anthesis in upper half of spike,
6) anthesis ended.

Each plant in the control strip at Lundin Links, in the garden and in the greenhouse, was scored weekly for six weeks for the number of inflorescences and the stage of flowering of each inflorescence. The mean stage of flowering was then calculated for each phenotype (as classified in June 1969) in each environment every week. Regressions of the mean stage of flowering against time revealed a difference of 8.1 days between the flowering times of the prostrate and erect phenotypes at Lundin Links.

From fig. 14 and table 11 (also Appendix 1, table 16, p 208), it can be seen that in all environments there are flowering time differences between the different phenotypes, with erect plantains flowering before prostrate plantains. Heterogeneity Chi^2 tests on numbers in each flowering stage on the last date of recording showed significant differences among growth habit types in the Lundin Links population ($P \leq 0.05$) and in the garden population ($P \leq 0.01$). Differences between the habit types in the greenhouse were not significant.

Table 11. Differences in flowering times between the phenotypes in different environments

Environment	Difference in mean flowering stage between prostrate and erect plants (expressed in days)
Lundin Links	1.11 stages = 8.10 days, (1 stage = 7.30 days)
Garden	0.14 stages = 1.03 days, (1 stage = 7.36 days)
Greenhouse	0.29 stages = 1.10 days, (1 stage = 3.79 days)

Assuming the garden and greenhouse to be relatively uniform environments, the results suggest that the differences in flowering time between the phenotypes may be partly genetic in origin. The environment at Lundin Links does play an important part in inducing these differences.

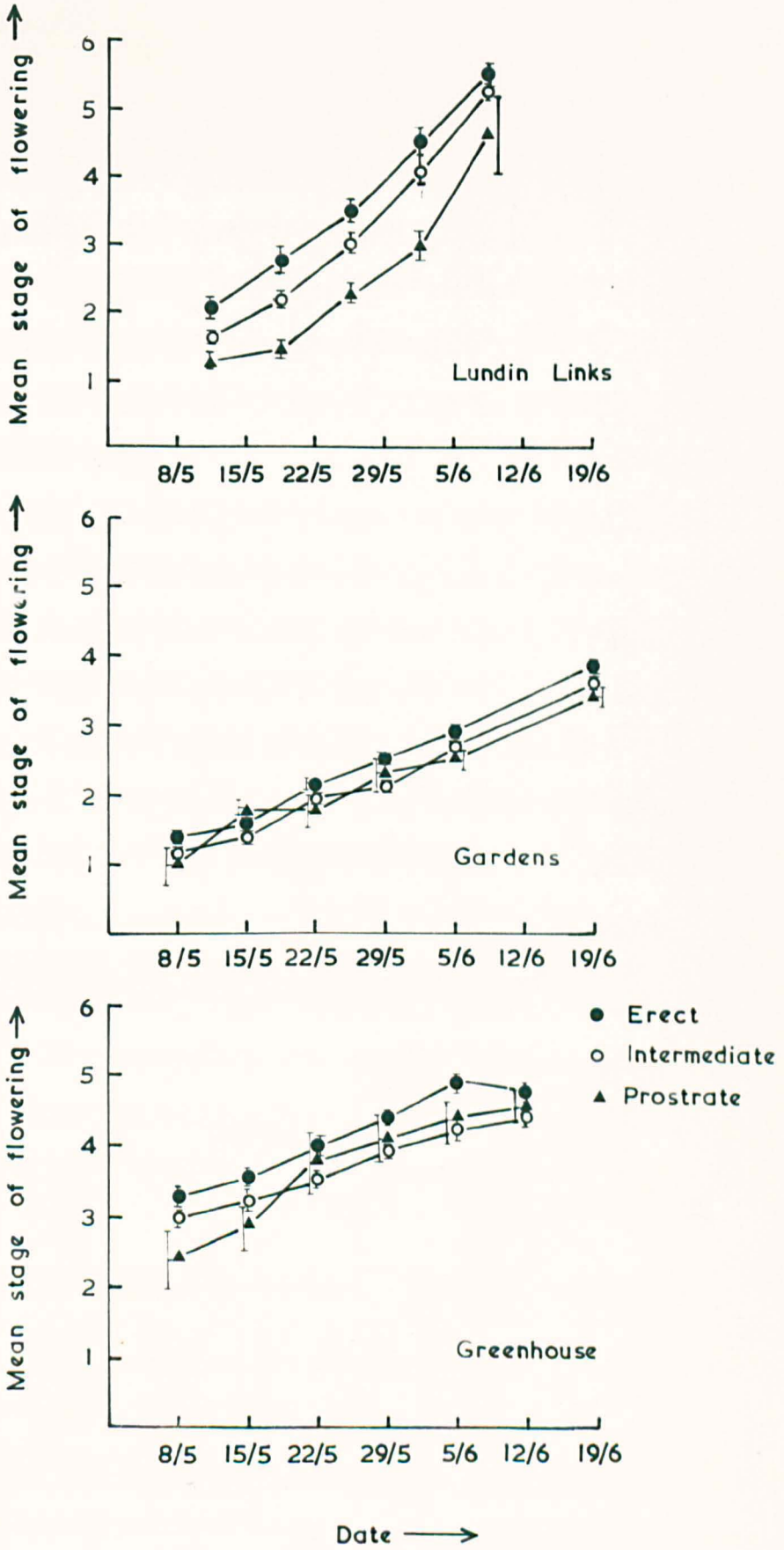


Fig. 14 Flowering time differences of prostrate, intermediate and erect phenotypes in the different environments.

Number of inflorescences produced

In June 1970, of the 196 plantains on the control strip at Lundin Links, 103 (or 52.6%) plants flowered. In the gardens, during the same period, 84/89 (or 94.4%) experimental strip plantains flowered indicating that the natural environment limits flower production. Also the flowering period at Lundin Links ceased at the beginning of July, whereas in the gardens it extended until September - probably a reflection of the effects of the competition pertaining in the dense sward at Lundin Links.

Comparison of the phenotypes (as distinguished by growth habit) at Lundin Links reveals that a lower proportion of the prostrate plantains produced flowers (table 12) compared with erect and intermediate types. However, this difference was not significant (Heterogeneity χ^2 = 0.97).

Table 12. The proportion of phenotypes which flowered in the different environments

Phenotype	Lundin Links			Greenhouse			Garden		
	No. plants	No. plants + flower	%	No. plants	No. plants + flower	%	No. plants	No. plants + flower	%
P	26	10	38	4	4	100	7	4	57
I	87	50	57	69	53	77	32	31	97
E	83	43	52	83	62	75	50	49	98

Key: P = prostrate growth habit; I = intermediate growth habit; E = erect growth habit

In the gardens, greenhouse and field, approximately equal proportions of intermediate and erect plants flowered although the percentages were different in the different environments,

indicating environmental effects. The trends, however, show that the prostrate plants in the field are limited in flower production in some way by their environment, compared with the other phenotypes. This could be due to the relatively exposed situation in which prostrate plantains are found (e.g. on the footpath) or selection for emphasis on vegetative rather than sexual reproduction. In the control and experimental strips in June 1969, a slightly higher proportion of prostrate plants were vegetatively propagated but this was not significantly different from the population as a whole. Table 13 gives the numbers of cloned plants found at Lundin Links.

Table 13. Proportion of cloned plants at Lundin Links, June 1969

	Growth habit:			
	P	I	E	Total
No. cloned plants	3 = 5.8%	5 = 3.3%	16 = 4.3%	16 = 4.3%
No. plants	52	154	168	374

Key: P = prostrate growth habit; I = intermediate growth habit; E = erect growth habit.

Therefore there does not seem to be selection for vegetative propagation and it is more likely that flower production by prostrate plantains is limited by the exposed situations in which they are found.

Amount of seed set

In order to estimate the number of seeds produced on the control strip at Lundin Links, seed heads (spikes) were collected and the number of seeds in each spike was correlated with the length of the spike ($r = 0.80$, fig. 15). (See also Appendix 1, table 17, p 209).

The length of the longest spike had been measured for each plantain on the control strip. Therefore from the correlation, the number of seeds per flowering plant was estimated. This may be an overestimate because it was assumed that all the spikes on an individual plant were the same length, i.e. as long as the longest spike. However, in the correlation no account was taken of any seeds which may have been shed before the seed head was collected.

Over the whole strip, i.e. the control strip, an estimated total of 2724 seeds was produced.

Comparison shows that different numbers of seeds were produced by the different phenotypes, prostrate plants producing the fewest seeds and intermediate plants producing the most (table 14).

Table 14. The estimated number of seeds produced by the phenotypes on the control strip

Phenotype	No. plants	No. plants + flower	Total no. seeds	No. seed per flowering plant	No. seed per plant
Prostrate	26	10	168	16.8	6.5
Intermediate	87	50	1517	30.3	17.4
Erect	83	43	1039	24.2	12.5
Total	196	103	2724	26.4	13.9

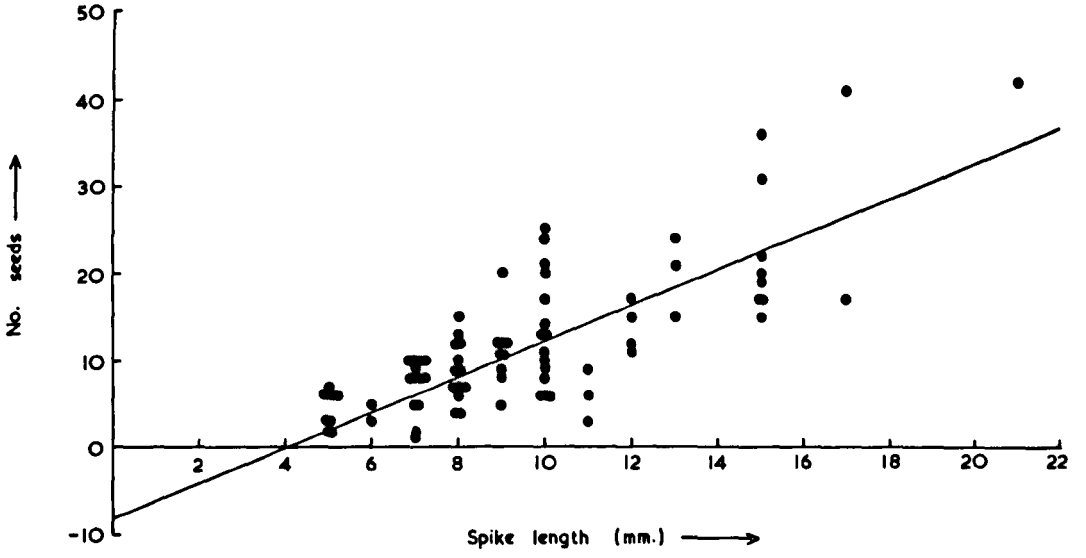


Fig. 15 Correlation between the number of seeds produced and spike length. (Correlation coefficient = 0.80, $P < 0.01$.)

The number of seeds produced has been shown to be proportional to spike length. It has also been found that spike length was significantly positively correlated with the growth habit of the plants on the control strip at Lundin Links (see results iia, p 50). Spike length was also found to be significantly correlated with growth habit after cultivation in the garden and greenhouse (see results iic, p 60) suggesting that the characters of growth habit and spike length (number of seeds) may be genetically linked.

b) Genetic variability and selection pressure

From the previous results (pp 50 - 68), which strongly suggest microadaptation within the small Plantago population, one must surmise that the selection pressures needed to maintain such a population would be quite high if there is a high degree of gene flow. It has been shown that there is some measure of reproductive isolation between the phenotypes (pp 69 - 72) and this would tend to reduce gene flow. Even so, with a precise match of phenotype and genotype to the environment (microadaptation), one might expect high selection pressures and the wastage of large amounts of seed. In order to estimate whether there was sufficient variability resident in the population to allow evolution to have proceeded in this way, a seed sample was taken at random from the general area at Lundin Links in July 1969. 208 seeds, spaced 2ft apart, were sown in a random block in the garden in May 1970. The plants were measured for leaf number, leaf width, leaf length, inflorescence number, scape length, spike length and growth habit in October 1970. Direct comparison of these plants with those of the experimental and control strips (in order

to obtain some estimate of the selection pressures) could not be made so they were compared with plants from the experimental strip growing under similar conditions (table 15). Unfortunately the conditions at the time of establishment and the initial development stage of the seed population and adult population were not the same, and the populations differed in their means: the seed population produced larger plants. Comparison of means was therefore clearly invalid, but comparison of variances was made subjectively using coefficients of variation.

Table 15. Comparison of cloned plants in the garden with general seed sample grown in the garden

Character	Expt[1]. strip plants in garden n = 86			General seed sample n = 200			
	Mean	Variance	Coeff. variation C_1	Mean	Variance	Coeff. variation C_2	$\frac{C_1}{C_2}$
Leaf no.	62.97	1057.31	0.52	100.84	3254.15	0.57	>
Leaf width (mm)	23.11	21.25	0.20	24.74	44.88	0.27	>
Leaf length(mm)	167.24	1084.39	0.20	365.31	5377.61	0.20	=
Inflo. no.	25.12	235.01	0.61	51.67	644.73	0.49	<
Scape length(mm)	326.66	5604.02	0.23	622.81	10681.53	0.17	<
Spike length(mm)	23.46	52.22	0.31	44.86	305.59	0.39	>
Growth habit*	1.92	0.31	0.29	2.18	0.81	0.41	>

* Growth habit calculated on a 1 - 3 scale.

It can be seen that the variance of the seed sample was largely equal to or greater than that of the experimental strip plants grown in the gardens (5/7 characters). It is evident

that sufficient genetic variability resided in the population, enabling natural selection to act as a sieve at each generation and perpetuate the extreme phenotypes.

c) Population turnover

As a further indication of the intensity of the potential selection pressures operating on the population, observations of population density, individual longevity, recruitment and mortality, decay rates and survivorship were made on the experimental and control strips. The emergent picture is of a population maintained by continuous selection and the processes of selection are highly dynamic.

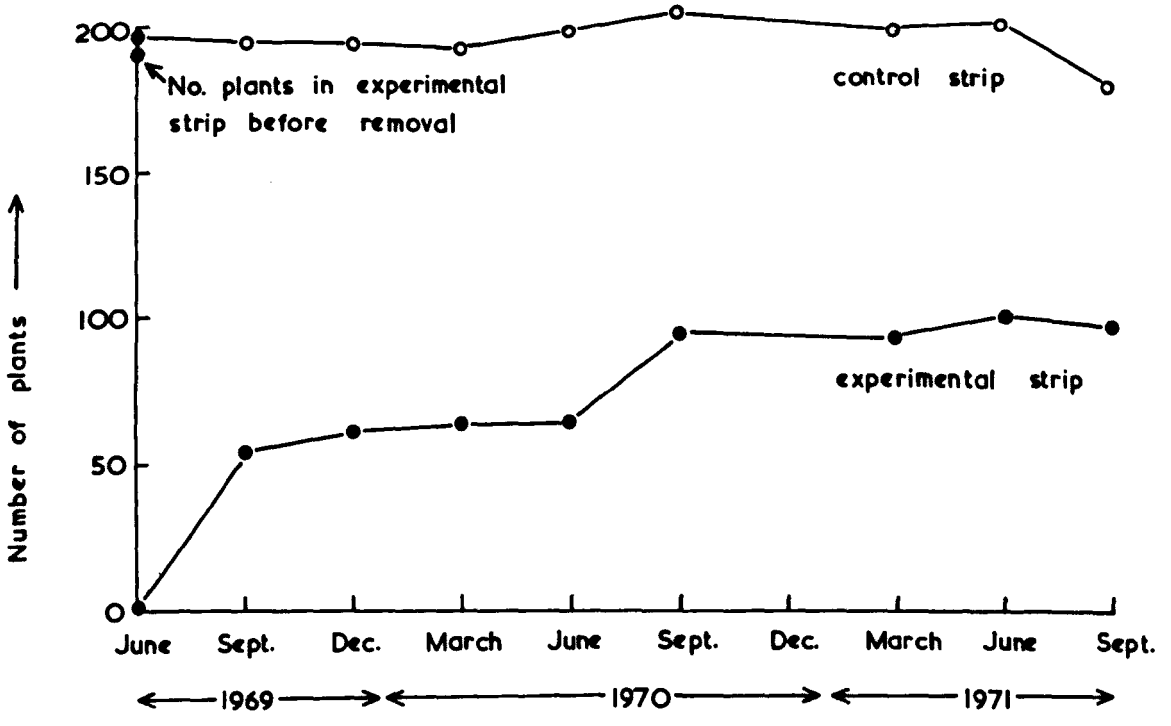
Population density

On the control strip, the total number of plants recorded on any occasion showed very little fluctuation, except for a decline in the last three months (fig. 16). On the experimental strip, the total number of plants which arrived after the initial population had been sampled, rose throughout the study period though the numbers never reached a similar level to those on the control strip. There was a rapid increase in the population density between June and September in 1969 and 1970 but this was not observed in 1971.

Individual longevity

On the control strip, only 80/365 plants lived throughout the study period (2 years). A large proportion of the short-lived individuals failed to flower and died within one year (fig. 17).

Fig. 16 Population density on the control and experimental strips during the study period.



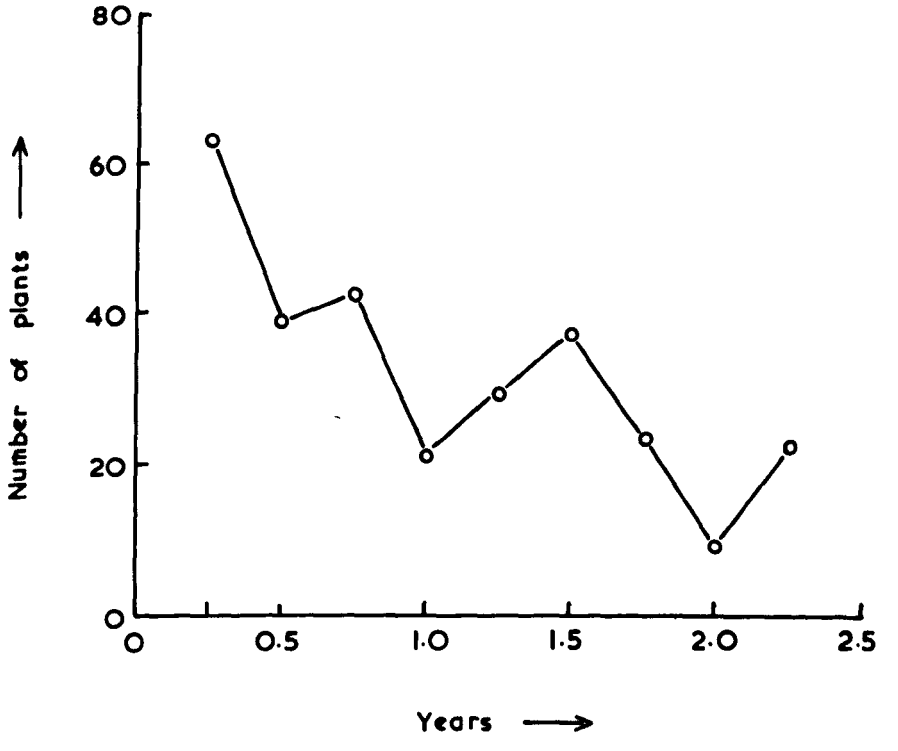


Fig. 17 Individual longevity of plants which died on the control strip during the study period.

Correlation of longevity with morphological characters of the plants in the control strip in June 1970 (table 16) shows that older plants tend to be larger in size than the short-lived plants. This is not surprising as one would expect plants which live longer to grow to a larger size. However, longevity is positively correlated with growth habit, indicating that erect plantains tend to live longer than prostrate plants.

Table 16. Correlation of longevity with morphological characters of plants in the control strip, June 1970

	Character						
	Leaf no.	Leaf width	Leaf length	Inflo. no.	Scape length	Spike length	Growth habit
longevity	0.18	0.36	0.38	0.04	0.26	0.18	0.22 (corr. coeff.)
	*	***	***		**		** (level of signif-icance)

Key: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$

Mortality and Recruitment

Mortality and recruitment were noted at every time interval throughout the study period. No clear seasonal trends were apparent (fig. 18, p 82, and table 17).

There seemed to be an association between mortality and recruitment: an increase or decrease in mortality in a 3-month period was paralleled by a corresponding change in recruitment. The association was nearly significant with $r = 0.67$; (for $r = 0.71$, $P \leq 0.05$). There was also a one to one correspondence in directional shifts in mortality and recruitment.

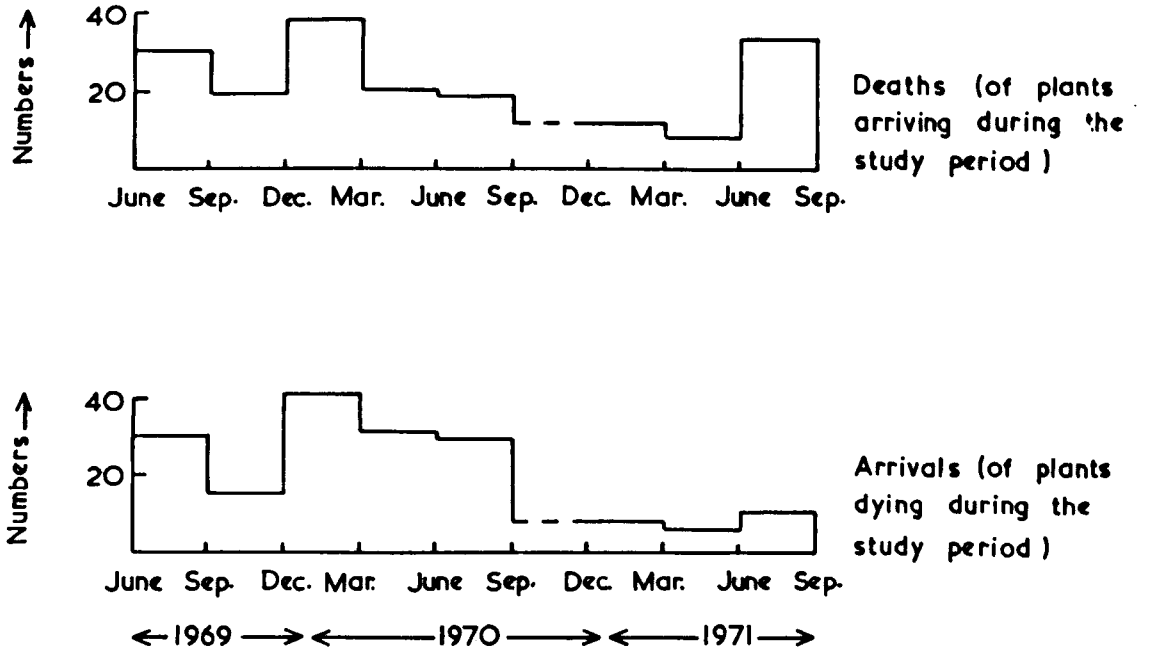


Fig. 18 Mortality and recruitment on the control strip at Lundin Links.

Table 17. Mortality and recruitment on the control strip, June 1969 - September 1971

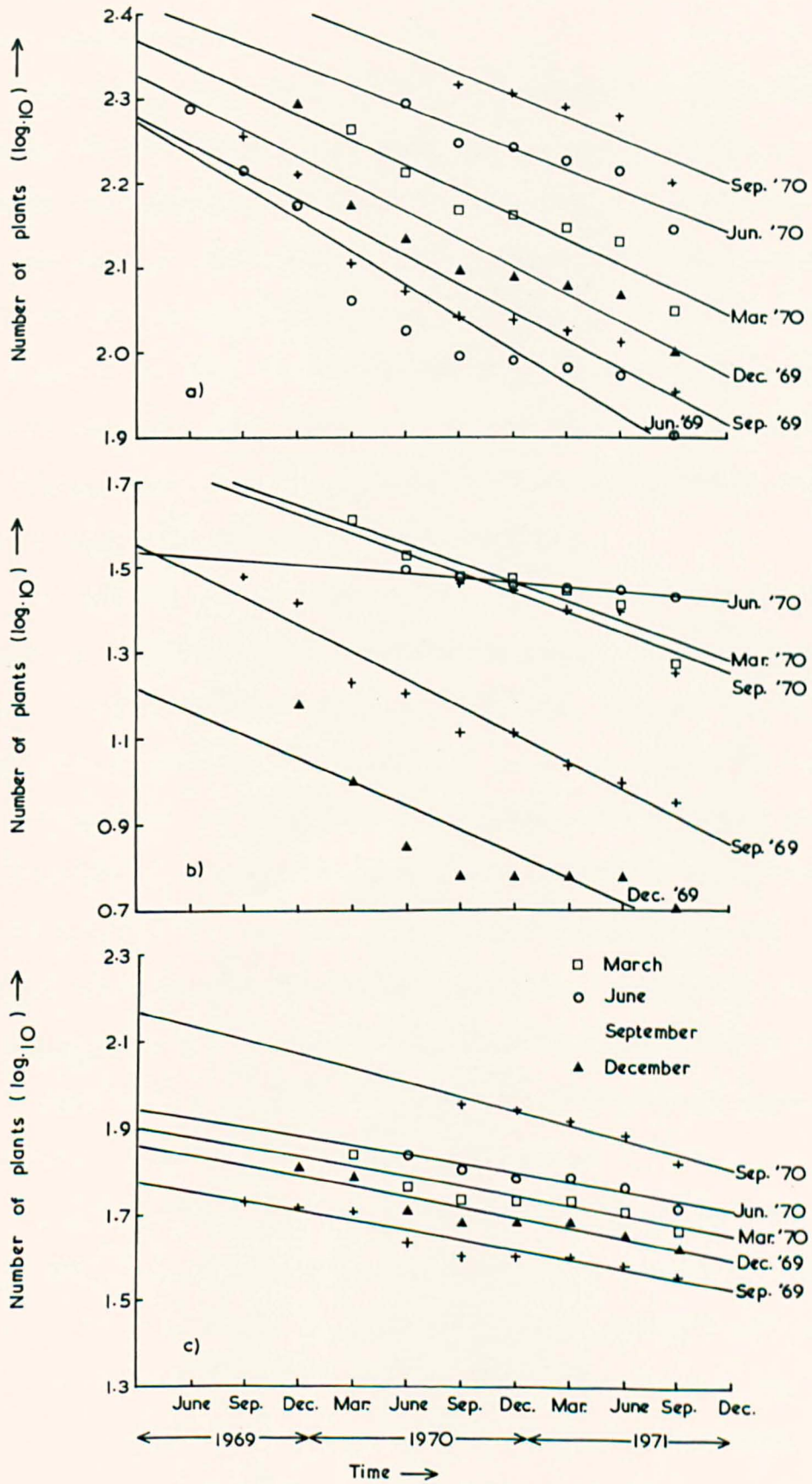
	1969				1970			1971		
	J-S	S-D	D-M	M-J	J-S	S-D	D-M	M-J	J-S	Total
No. deaths in time interval	30	19	48	20	18	-	11	7	33	186
No. births in time interval	30	15	41	31	29	-	7	5	10	168
Directional shift	-	+	-	-		-	-	+		

Key: M = March; J = June; S = September; D = December

Population decay rates and survivorship

The decline in numbers of rosettes present at the beginning of successive time intervals (3 months) of the study was plotted on a log scale and regression lines fitted (Appendix 1, tables 18a, b and c, p 210). When the whole population is considered (fig. 19a, p 84) i.e. individuals starting in one year are included with those established in previous years, the rate of decay remained more or less constant with time. The regressions did not differ significantly in slope ($P < 0.55$). The overall regression gave a population half life of 2.17 years. When the course of population decay was plotted for new arrivals at each time interval (fig. 19b), the curves were generally linear except for those of plants establishing in December 1969, where there was a rapid decay rate for the first six months. The regressions also differed significantly in slope, ($P < 0.02$), with the plants establishing in June 1970 having a much slower decay rate. The half life of the June 1970 new arrivals was 8.66

Fig. 19 Population decay curves at Lundin Links during the study period; a) whole population, b) new arrivals on the control strip, c) new arrivals on the experimental strip.



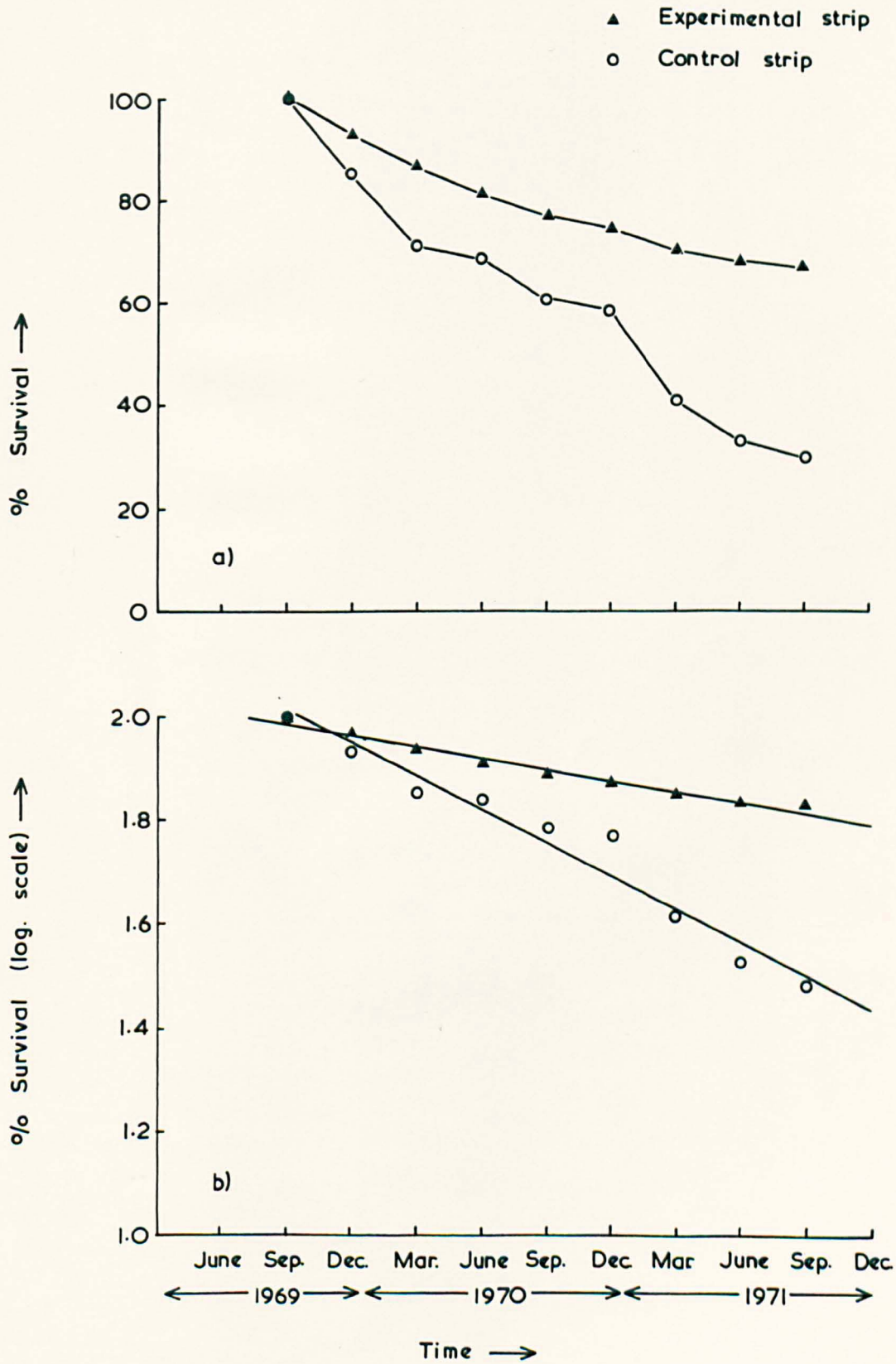
years compared with a value of 1.44 years for all new arrivals on the control strip.

The fate of new arrivals in the experimental strip (after removal of all plants at the start of the investigation as part of the genecological sampling) was also followed (fig. 19c, Appendix 1, table 18c, p 210). Plants arriving at different dates did not have significantly different decay rates, ($P = 0.67$); the decay rates were remarkably constant and independent of the time of arrival. The overall decay rate of the new arrivals on the experimental strip gave a half life of 3.47 years, a much slower decay rate than on the control strip.

In order to compare more clearly the survivorship of the plants on the control and experimental strips, mean survivorship curves (of % survival plotted against time) over all arrival dates were calculated and plotted on a linear as well as log scale (fig. 20a and b, p 86). As the ages of the plants already established on the control strip were not known, the survivorship curves are for the survival of new individuals and do not include the period before establishment of the plants as recognisable P. lanceolata. The results clearly show that the survival of the plants on the experimental strip was consistently greater than that of the plants on the control strip.

A more complete picture of survival in the pasture at Lundin Links comes from the observations on spike length; these were used to estimate the total amount of seed produced by the plants on the control strip in June 1970 (see results iiia, p 75, for full details). It was shown that there were approximately 2724 seeds produced by the population. Since there were 51 new arrivals

Fig. 20 Survivorship curves of the new arrivals on the control and experimental strips at Lundin Links.



in the following season (to June 1971) it was estimated that only 1.87% of the seeds developed into recognisable adults. The entire survivorship curve is therefore almost certainly very concave, with a very high juvenile mortality followed by a more or less constant probability of death at subsequent ages.

d) Discussion

The population of P. lanceolata growing at Lundin Links showed a very rapid turnover. Less than half of the plants lived for more than two years, and correspondingly the population half life was a little over two years, comparable with the half life of 3.2 years noted by Sagar (1959) for a P. lanceolata population in a 'permanent' pasture in Oxford. This suggests that the habitat-correlated genetic differences reported in results ii, pp 60 - 68 are maintained by continuous selection, and not simply by the long term accumulation of best adapted genotypes. The processes of selection are therefore highly dynamic. Quantitative coefficients of selection were not available from this investigation although it was shown that sufficient genetic variability existed in the Plantago population to support strong selection pressure. In a more recent study of Anthoxanthum odoratum growing in a park grass environment, patchy by virtue of different fertilizer treatments over many years, Davies and Snaydon (1976) have revealed the operation of very high selection pressures. The population of A. odoratum has a half life of approximately 2 years, which is comparable to that pertaining in the present investigation. Therefore one might suppose that equally strong selection pressures may be operating on the Plantago population.

The processes of population regulation in this population may also be highly precise. Throughout the study the population density in the control strip did not vary greatly. However, when population density was artificially lowered by removing the plants from the experimental strip, the population size in this area increased gradually over the study period. The population control was also highly localised: the removal of plants in the experimental strip did not detectably influence the population in the control strip. This, together with the findings of Putwain, Machin and Harper (1968) that deliberate sowing of Rumex acetosella seed at different densities on a sward had no effect on the Rumex population in the following spring, indicates the precision of the mechanisms of population control in plants.

Evidence as to the nature of population control comes from the associated data. Within each three-monthly time interval, mortality rates closely paralleled recruitment rates both in magnitude and direction. Similar results have been obtained by Antonovics (1972) and Sarukhán and Harper (1973). These observations suggest that many plant populations (particularly perhaps in pastures) have the ability to detect recruitment and/or mortality and can respond appropriately by corresponding shifts in mortality and/or recruitment. The analysis of decay rates and survivorship curves suggests that the populations are unlikely to be limited by seed supply: numerous seeds were produced and relatively few survived. In the present study there were probably also numerous seeds available to the experimental strip from the control strip (plants on the 'outside' of the experimental strip were often heavily damaged by trampling

during measurement and mapping). Although there was a slightly greater relative concentration of new arrivals in the experimental strip in the half of the quadrat nearer the control strip, this greater concentration was not significantly different ($\chi^2 = 0.62$) from that present at the time of sampling. It appears therefore that availability of seed was not limiting in these densely populated areas. There is considerable evidence however, that seedling establishment may have been a severe limitation, especially in areas of low density. Evidence for this comes from the fact that the population on the experimental strip was not restored to its original density in one season: even after two and a half years the population density had only reached a little over half its original value. Further evidence of problems during establishment comes from the survivorship of plants that established at different times. Plants establishing in December 1969 showed an initially high mortality and one could speculate that in establishing at this time of year they had not accumulated sufficient resources to survive well in the following spring, when competition by vigorously growing surrounding plants increased. Conversely, plants establishing in June 1970, most of which may have established under particularly favourable conditions, showed a high survivorship. More generally, the observation that new arrivals had a greater decay rate on the control strip than the overall populations, suggests that young individuals were more susceptible to environmental hazards than older ones. Correlation with field phenotypes confirms that younger plants were morphologically smaller.

Evidence of control by competition between adult individuals at high density comes from observations on the decay rates on

the experimental strip. These are considerably lower than the values on the control strip both for established individuals and for new arrivals. This suggests that density is an important determinant of mortality, being higher at higher densities.

The overall pattern of population control that emerges is of a steady input of seeds, each with a low probability of establishment. Establishment itself appears to depend on the environmental density of surrounding plants of the same species and probably also different species and on the interaction between the two. Increasing densities of plants of the same species result in a lowered survivorship of existing individuals as well as of newly established seedlings.

Much of the reason for the high turnover and mortality may be the selection process itself. We can recognise two, albeit arbitrary 'components' constituting the seeds entering any micro-habitat, namely, those seeds which are adapted to that niche and those that are not. Clearly there will be a continuum between these two categories. Given that there is a finite chance of the seeds of both classes becoming established, there will be mortality due to selective causes acting on the non-adapted component, and mortality due to 'chance' effects (disease, changes in micro-habitat, predators, etc.) acting on both adapted and unadapted types. The balance between these various forces and their relative magnitudes will determine the rate of population turnover. This will be relatively constant in a well-established population, where equilibrium between the various population influences is likely to have been attained.

4. General Discussion

The existence of different phenotypes is a well-documented phenomenon for P. lanceolata (Pilger 1937; Böcher 1943; Sagar and Harper 1964) and for P. maritima (Gregor 1938). That the different forms are phenotypically suited to their environment, with, for example, prostrate plants being found in open situations and erect plants amongst taller vegetation, is also not surprising. The present study shows that such phenotypic adaptation can readily occur within a small area, particularly if the micro-habitats are sufficiently different. In the light of recent research, it is just possible that these differences may be attributable to direct environmental induction of heritable changes as, for example, in Linum usitatissimum (Durrant 1962) or, as in Lolium perenne, to the modifying effect of an extra-nuclear component (Hayward and Breese 1968). However, the environmentally-induced changes observed by Durrant are peculiar to one variety of flax and the findings of Hayward and Breese are not strictly applicable to P. lanceolata. L. perenne is extensively reproduced by vegetative propagation. Hence the transmission of induced heritable extra-nuclear components throughout successive 'generations' is a possible strategy for the population to adopt if it is in a rapidly changing environment. The induction of heritable changes in a readily-cloned plant may be more efficient than the exploitation of genetic variability by sexual reproduction. Since P. lanceolata is self-sterile and, in this study, cloned plants formed only four per cent of the population, it is more likely that the phenotypic differences observed are genetic

and not environmentally-induced. The plantains investigated under cultivation were cloned from small propagules and there were considerable changes in the plant size in the different environments. The study of the different plantain populations also demonstrated the existence of genetic variability of plant size. Comparison of the cloned plants in the different environments shows that some characters are heritable and that, in most cases, there is both a genetic and environmental component in the observed adaptation. Often the genetic differences are subtle in that they are not absolute but manifest themselves as differences in response or phenotypic plasticity. The greater response to shading of prostrate populations demonstrated in ii:2, p 13, testifies to the fact that there is probably selection for phenotypic plasticity as postulated by Bradshaw (1965) and this would be a good strategy to adopt in response to seasonal variation. Clearly, further study is needed, particularly experiments in which the inheritance of differences through the seed is studied, but even so, the weight of evidence presented above suggests that the underlying differences are genetic.

The present study was undertaken largely to investigate whether genetic differences could occur in a heterogeneous environment on a scale well within the pollen and seed dispersal range of a species. There appears to be no field data on the range of pollen and seed dispersal in P. lanceolata but this has been shown to be very localised in Phlox (Levin and Kerster 1968). Smith (1966) discussed the theoretical liklihood of sympatric speciation and Bradshaw (1972) has also argued the feasibility of such small-scale microdifferentiation. There is considerable

evidence of differentiation in parapatric situations. Genetic differentiation at abrupt boundaries of habitats has been demonstrated most clearly by Bradshaw and his associates (Jain & Bradshaw 1966; Aston & Bradshaw 1966; McNeilly 1968; McNeilly & Antonovics 1968; Antonovics & Bradshaw 1970). Gradual changes in the environment have been reflected in clinal patterns of population differentiation, as in P. maritima (Gregor 1930). Snaydon (1970) demonstrated small-scale clinal differentiation in A. odoratum. Therefore such differentiation can no longer be regarded as unexpected. The most convincing evidence that adaptation can be extremely precisely matched to the heterogeneity of the environment comes from the work of Snaydon. He showed that i) plants of Agrostis canina and Festuca ovina growing directly below a galvanised (zinc coated) fence were tolerant to enhanced levels of the metal, whereas plants a few inches away from the fence were not (reported in Bradshaw, McNeilly & Gregory 1965); ii) a distance of less than 150mm separated contrasting populations of Anthoxanthum odoratum growing on soils given different fertilizer treatments over a period of 50 years at Rothamsted (Snaydon 1970). It therefore seems that the pattern of differentiation does indeed follow the pattern of the environment fairly closely. In a computer simulation (Dickinson & Antonovics 1973b) based partly on the field study in this thesis, it was shown that this may be the case - a heterogeneous habitat could maintain genetic variability and lead to a significant genotype environment correlation if selection was sufficiently severe, even if there was complete random breeding among the genotypes in the habitat. It therefore seemed appropriate to test the possibility of micro-differentiation within a putatively random

breeding population. Plantago lanceolata was chosen for the reasons outlined in section II:1. The use of this particular species added another dimension to the study. As has been mentioned above, P. lanceolata is very flexible phenotypically: it might be expected that the plant may undergo local adaptation purely by phenotypic response. For example, Marshall and Jain (1968) have shown that Avena fatua and Avena barbata have alternative strategies of adaptation, with one species (A. fatua) being primarily genetically variable and the other species being more flexible phenotypically. Clearly, phenotypic and genetic variability may play different roles in adaptation depending on the scale of heterogeneity (Levins 1964).

The present study shows that in fact microdifferentiation within plant populations in response to habitat heterogeneity may be commonplace. The area chosen for the study was not particularly extreme. Moreover it is clear that even though the species or population may be phenotypically very flexible, it is still 'advantageous' for the population to be genetically adapted to the niches within its habitat. There may be conflicting pressures on the population in this regard. It is probably advantageous to be flexible, since individuals once established could cope readily with changes in the environments surrounding them; the overall genetic load on the population would be less and it may be a means of avoiding genetic segregation that could break down character complexes. On the other hand, there is presumably some cost to being phenotypically flexible: either the metabolic efficiency of all-purpose genotypes may be less or the time lag involved in response may be too slow to track the environmental changes. Wright (1951) postulated that

microdifferentiation would lead to an increased fitness of the population as a whole thus favouring genetic adaptation.

The existence of habitat correlated genotypes is evidence that the intensity of selection is very strong: genetic variation is found in several characters that do not show habitat correlation. This may be due to selection pressures being sufficient to maintain the variation yet not being sufficiently severe to establish a correlation: in such cases the reasons for the existence of genetic variability may include the possibility of linkage or developmental correlation. In the present study, the major environmental factor investigated was vegetation height. Phenotypes (and by inference genotypes) differing in their growth habit were associated with different species, so biotic interactions may also be important. Again it is not clear exactly what environmental factors are correlated with the height differences observed: there are probably many selective influences on the population.

Even if one concentrates on the single factor of height, further examination of the results reveals that its action is complex. It seems that in low vegetation selection operates on the seedling stage, resulting in high seedling mortality. In the taller vegetation, fewer seedlings become established initially but survival is better. In the taller vegetation therefore, selection may operate before seedling establishment. Population density and seedling establishment are greater in the regions of short vegetation, indicating that these areas support a greater density of individuals, and that selection is probably not directly related to population size. In terms of reproduction, however,

most of the seeds on a total basis and on a per plant basis are produced by erect and intermediate plants in the taller vegetation. It might be predicted from the seed data that the erect and intermediate genotypes would come to predominate in the population, but the data provide a reason why this is not the case. The density of plants in the different micro-habitats appears to be independently controlled: there are large consistent year to year differences in density between the quadrats, population control is very precise, and population control is very localised in terms of distance. Theoretical models confirm that a pre-requisite for microdifferentiation in a heterogeneous habitat is that the population size in the different habitats is controlled independently of the selective forces operating (Smith 1966; Dickinson & Antonovics 1973a and b).

Other factors are probably present promoting the micro-differentiation. There is evidence that gene flow between the plants is restricted by differences in flowering time of different phenotypes and genotypes differing in growth habit. P. lanceolata is self-sterile and largely wind pollinated (Sagar & Harper 1964) and therefore flowering time differences would be very effective in reducing gene flow. Sagar and Harper (1964) report that the period of flowering of P. lanceolata is approximately twelve weeks over June, July and August but may finish by early July if competition is severe. At Lundin Links, the flowering period extended for approximately eight weeks but pollen production was for only six weeks of that time. A difference of 8.1 days between prostrate and erect plantains at the beginning of anthesis (see results iiia, p 71) would represent isolation of $\frac{8.1}{42} \times 100$ = approximately 20% of the plants, which would be important in

maintaining the genotypic differences noted in the heterogeneous habitat. Differences in flowering time between Anthoxanthum odoratum plants situated at the boundaries of the patchy environment at Rothamsted have also been observed by Snaydon and Davies (1976). It is also known (Sagar & Harper 1964) that older plants flower before younger plants. One reason for the earlier flowering of the erect plants in the field may be that they are older than the prostrate plants: evidence for this comes from the fact that turnover in low vegetation, where the plants are predominantly prostrate, is higher than in the taller vegetation. Alternatively, the differing environmental conditions around prostrate, erect and intermediate genotypes may induce the larger differences in flowering time observed at Lundin Links. The garden population does, however, display flowering time differences, though to a lesser extent, indicating either a genetic or permanently induced component in the variation.

The studies on population turnover strongly support the idea of severe selection pressures and indicate the nature of the factors which are regulating population size in P. lanceolata. These factors seem to act chiefly but not exclusively at three levels: at very low densities availability of seeds may be limiting (but this was not demonstrated); at intermediate densities the crucial factor is rate of seedling establishment; and at high densities such as were normally found on the Lundin Link site, interplant competition seemed to be the most important.

It would therefore appear that strong selection pressures may combine to operate in an ostensibly 'ordinary' heterogeneous

environment, such as dune pasture, giving rise to micro-differentiation on a very small scale. This study therefore contributes to the growing evidence that such microdifferentiation in plant populations may be commonplace.

5. Summary and Conclusions

1. An investigation was carried out on the morphology and response to shading of five discrete populations of Plantago lanceolata. Distinct population differences were found, although each population comprised a gradation of phenotypes, from prostrate rosettes with shorter leaves and inflorescences to erect rosettes with larger leaves and inflorescences. The two populations used for the response experiment showed differences in phenotypic flexibility.

2. The major investigation was to study microdifferentiation in a population of P. lanceolata occupying a small area of dune pasture, heterogeneous by virtue of different vegetation heights and differing vegetation.

3. The population was found to consist of a gradation of phenotypes and these phenotypes were correlated with the environment, prostrate forms with shorter leaves and inflorescences being associated with low vegetation and erect forms with larger leaves and inflorescences being associated with taller vegetation.

When half of the plants were removed to the standard environmental conditions of garden and greenhouse, persistent genotype differences of leaf length, scape and spike length and growth habit were noted. Of these, leaf length and growth habit (the two most noticeable phenotypic characters) were still correlated with the original environment of the plants, thus indicating microdifferentiation of the population; some measure of phenotypic flexibility was also apparent.

4. Simultaneous investigation of the population parameters pertaining to the other half of the population left *in situ* revealed some reproductive isolation between the phenotypes - erect plants flowering before prostrate plants - and the erect plants produced more, larger inflorescences which set more seed.

5. The population left *in situ* showed a half life of a little over two years. There was a rapid turnover of individuals less than six months old, particularly in low vegetation where the population was densest, and relatively little establishment of new individuals in tall vegetation, indicating intense selection pressures operating independently in different habitats of the heterogeneous environment. Despite this, population numbers remained relatively constant, mortality being matched by recruitment, thus indicating precise population control. The chances of survival of an individual were little affected by the season of establishment but were increased in the experimental strip where the population density had been reduced to zero.

6. It was concluded that genetic microdifferentiation, as a result of severe selection pressures operating in a heterogeneous environment, may be a common phenomenon in plant populations.

SECTION III.

DROSOPHILA MELANOGASTER IN A HETEROGENEOUS ENVIRONMENT

SECTION III

DROSOPHILA MELANOGASTER IN A HETEROGENEOUS ENVIRONMENT

CONTENTS

Page no.

"(Morgan's) . . . subject for investigation has been a small parasitic fruit-fly, Drosophila melanogaster, of which it has been said that it has been created by God solely as an object of heredity research".

Nordenskjöld: The History of Biology.

1. Introduction

For reasons first appreciated by Morgan and endorsed by later geneticists, D. melanogaster was used as the experimental subject for this attempt to demonstrate sympatric divergence in a laboratory population.

In a series of laboratory experiments with Drosophila, Thoday and his colleagues (Thoday 1959, Thoday & Boam 1959, Millicent & Thoday 1961, Thoday & Boam 1961, Gibson & Thoday, 1963, 1964) demonstrated increasing bimodality in a continuously varying population due to disruptive selection. Selecting for 'high' and 'low' sternopleural chaeta numbers in each generation and even enforcing 50% gene flow between the two lines, they found divergence between 'high' and 'low' lines. Unsuccessful attempts to repeat this experiment using different strains of Drosophila, (Chabora 1968, Scharloo, van Boer & Hoogmoed 1967, Barker & Cummings 1969), have suggested that the response to disruptive selection in the face of gene flow is not an automatic response to disruptive selection and that additional genetic factors may be involved. In the ensuing controversy, as yet unresolved, questions have been raised on the extent of genetic variability within Thoday's original stock and on the extent of competition between the divergent lines. Scharloo (1971) maintains that is is possible that the stock was divergent before the experiment began and that disruptive selection merely increased

the isolation within the population and did not originate it - a necessity in any demonstration of sympatric divergence. However, Thoday and Gibson (1970) conclude from their experimental investigations that given the favourable conditions of a highly variable Drosophila stock, a high heritability of the character under selection with some means of frequency dependence of the two extremes, divergence under disruptive selection would be possible.

The disruptive selection and the mating schedules in all the above experiments were imposed by the experimenters. A more naturalistic approach was attempted by Robertson (1966). He was not concerned with trying to demonstrate the origins of divergence but attempted to demonstrate reproductive isolation in a divergent population in a spatially heterogeneous environment. A Drosophila population was allowed to adapt to a semi-toxic food medium containing EDTA. Migration was then allowed between this population and the base population - adapted to ordinary food - in the presence of both foods. No reproductive isolation or mating preference became apparent, although hybrids between the two populations were not well adapted to either medium.

Theoretical models of sympatric divergence (see p 3) demand that the selection pressures operating on the niches within a heterogeneous environment be high and independent of each other. In the following attempt to demonstrate sympatric divergence within a population living in a heterogeneous environment and allowed to mate at random, the disruptive selection was provided by two different food media, normal food and normal food plus peppermint, which is toxic in high concentrations. The flies

were not pre-adapted to this peppermint food. Unlike the imposed selection in Thoday's experiments, the selection pressures in this model were not maintained but would decline as the population adapted to the food. The base population was presumed to be adapted to the normal food but unadapted to the peppermint food. Thus disruptive selection on the population in the heterogeneous environment would be provided by a force of directional selection on the toxic food and by another force, not necessarily directional, on the normal food.

The flies used in the experiment were CB7 stock, descended from one inseminated female caught in Bangor, N. Wales, in 1965. This stock has had a relatively short history of laboratory culture and Antonovics (pers. comm.), using a recognised test of outbreeding, has shown that sternopleural chaeta number is a highly heritable and variable character.

The major problem presented by the experiment is the difficulty of observing divergence within a population when the character under selection varies continuously. Flies adapted to a toxic food need not look any different from those adapted to normal food. As demonstrated experimentally by Ayala (1968) and Beardmore, Dobzhansky and Pavlovsky (1960) and theoretically by Clarke (1972), a population consisting of different phenotypes, each fitted to a different niche in a heterogeneous environment, would be 'larger' than a monomorphic one. Therefore, in the main experiment, the population size (number of emergent progeny) in the heterogeneous environment was compared with that of the control populations - an increase in size indicating divergence. It became apparent during the experiment, however, that the number

of emergent progeny was dependent not only on the experimental variables of the two food media but also on the average weight of the flies within the population. This, in turn, was dependent on the density of the parental population (Shorrocks 1970). Therefore, biomass (number of emergent progeny x average weight per fly) represented a better measure of the true 'size' of a population in response to the experimental heterogeneous situation and was measured in later tests.

It was further necessary to the demonstration of sympatric divergence to show that any change in population size in the heterogeneous environment was a genetic response and not merely an environmental response. Therefore, tests of genetic adaptation to both the peppermint food and the heterogeneity were carried out. The population was also tested for other evidence of a response such as habitat selection. This would increase the chance of divergence and reproductive isolation (Smith 1966).

2. The Response of D. melanogaster to peppermint

In the main Drosophila experiment, the heterogeneity was to be provided by the food. A previous small-scale experiment on sympatric divergence (Threlfall, unpubl. data) had yielded promising results using a normal food medium (Mittler & Bennett 1962) and a toxic food medium - this same agar, sucrose and yeast food impregnated with peppermint. It was decided that these media should again be used. Preliminary experiments were therefore conducted (1) to find a suitable concentration of peppermint and (2) to test the immediate effect of this medium on the flies - the base population with which the main experiment was to be started.

2:1 Investigation of the Toxicity of Peppermint to D. melanogaster

Materials and Methods

3" x 1" vials containing 10ml of food medium (Mittler & Bennett 1962; see Appendix 2, p 212, for preparation) with 0, 0.03, 0.04, 0.05, 0.07, 0.10, 0.30, 0.50, 0.70, 0.90 and 1.00% peppermint essence added at 60°C were set up with 5 replicates of each concentration. (Essence of peppermint B.P.C. - a 10% solution of peppermint oil (chiefly menthyl acetate, menthol and terpenes) in 90% ethyl alcohol supplied by Boots Pure Drug Co. Ltd. was used.)

4 females and 2 males from stock CB7 (see Appendix 2, p 213, for history and life cycle) were introduced into each of the

vials, which were then incubated at 25°C. The parents were removed after 6 days, deaths being noted. Ether was used as the anaesthetic (see Appendix 2, p 214). The progeny were counted each day after emergence until the 23rd day after the parents were introduced, that is, until the 12th day after first emergence at which time a second generation would begin to emerge.

Results

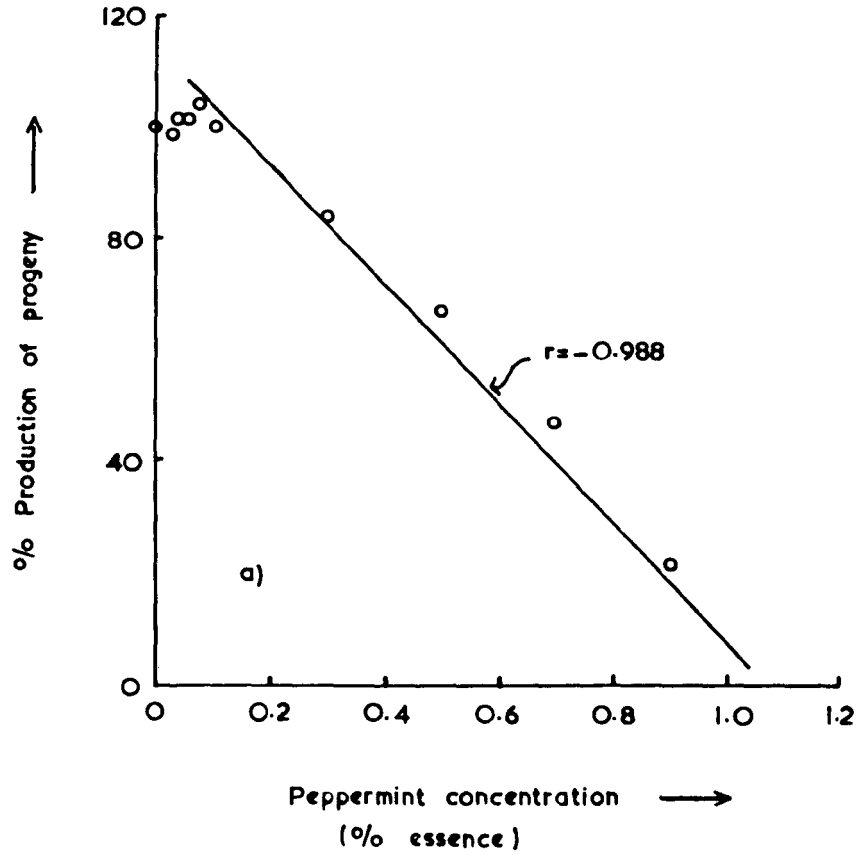
i) Number of emergent flies

As the peppermint concentration in the food increased, the number of flies emerging by day 23 decreased (correlation coefficient $r = -0.977$, $P < 0.001$). In fig. 21a (see Appendix 2, table 1, p 215 for data), the number of emergent flies by day 23 is expressed as a percentage of the flies emerging on 0 peppermint food and a regression line is drawn in the range of 0.1 - 1.0% peppermint essence inclusively.

For the main heterogeneity experiment, choice of a suitable peppermint concentration necessitated that at least 60% production of progeny was achieved in order to obtain sufficient flies to form a second generation. It was estimated from a probit analysis of the results that this production would be obtained using food containing 0.5% peppermint essence.

ii) Mortality of parents at 6 days

The % mortality of the parents increased as the concentration of peppermint increased until at a level of 1.00% peppermint essence all the adults were killed (fig. 21b and Appendix 2, table 2, p 216). It is noticeable that the males were more susceptible



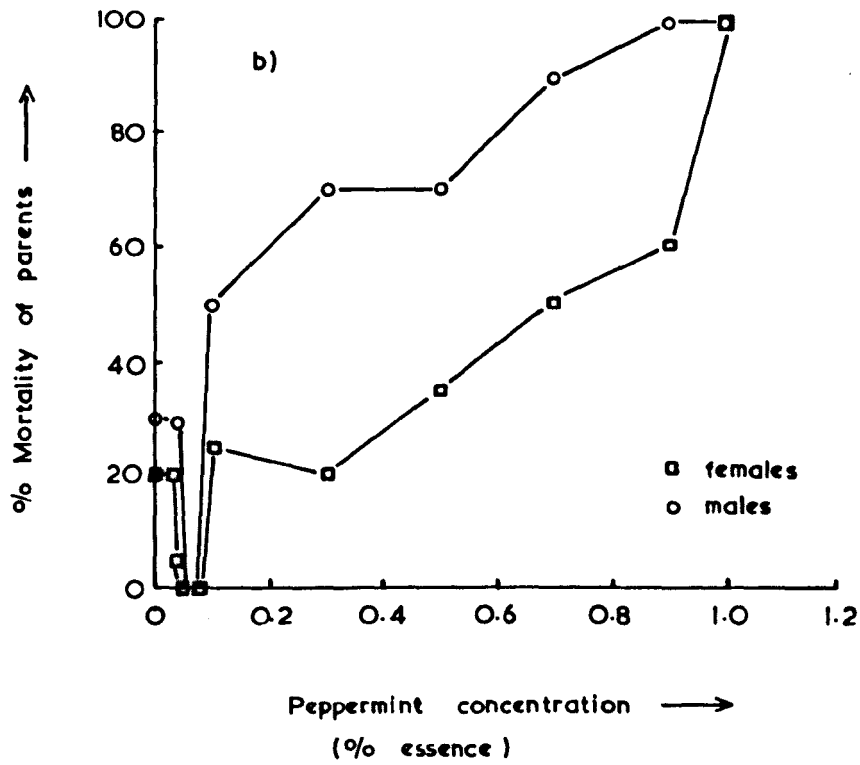


Fig. 21 a) The effect of peppermint on the yield of D. melanogaster

b) Mortality of parents at day 6. (Standard errors in table 2, appendix 2, p 216).

to peppermint toxicity than the females.

It is concluded, therefore, that peppermint has adverse effects on both adult flies and on the number of their progeny and that the toxic effect of peppermint is directly related to the dosage.

2:2 Investigation of the Vulnerability of Different Stages of the Life Cycle to Peppermint

Materials and Methods

Eggs, larvae and pupae were transferred from normal medium to 3" x 1" vials containing 0, 0.3, 0.5, 0.7 and 0.9% peppermint food. There were 4 replicates of each. 20 individuals at the same stage were placed in each vial and incubated at 25°C. The vials were scored for the number of individuals surviving to the next stage. Vials of the same range of peppermint concentrations were also set up with 7 virgin females and 3 males. These were scored for the number of eggs laid. The adults were at a different density from the eggs, larvae and pupae, since it was felt that the vials would be too overcrowded if 20 females were used.

Results

Increasing amounts of peppermint appeared to have no effect on the survival of eggs, larvae and pupae (fig. 22a; Appendix 2, table 3, p 217 for data). It can be seen from the survival of approximately 100% of the larvae and pupae that they were also unaffected by the transfer. The survival of the transferred

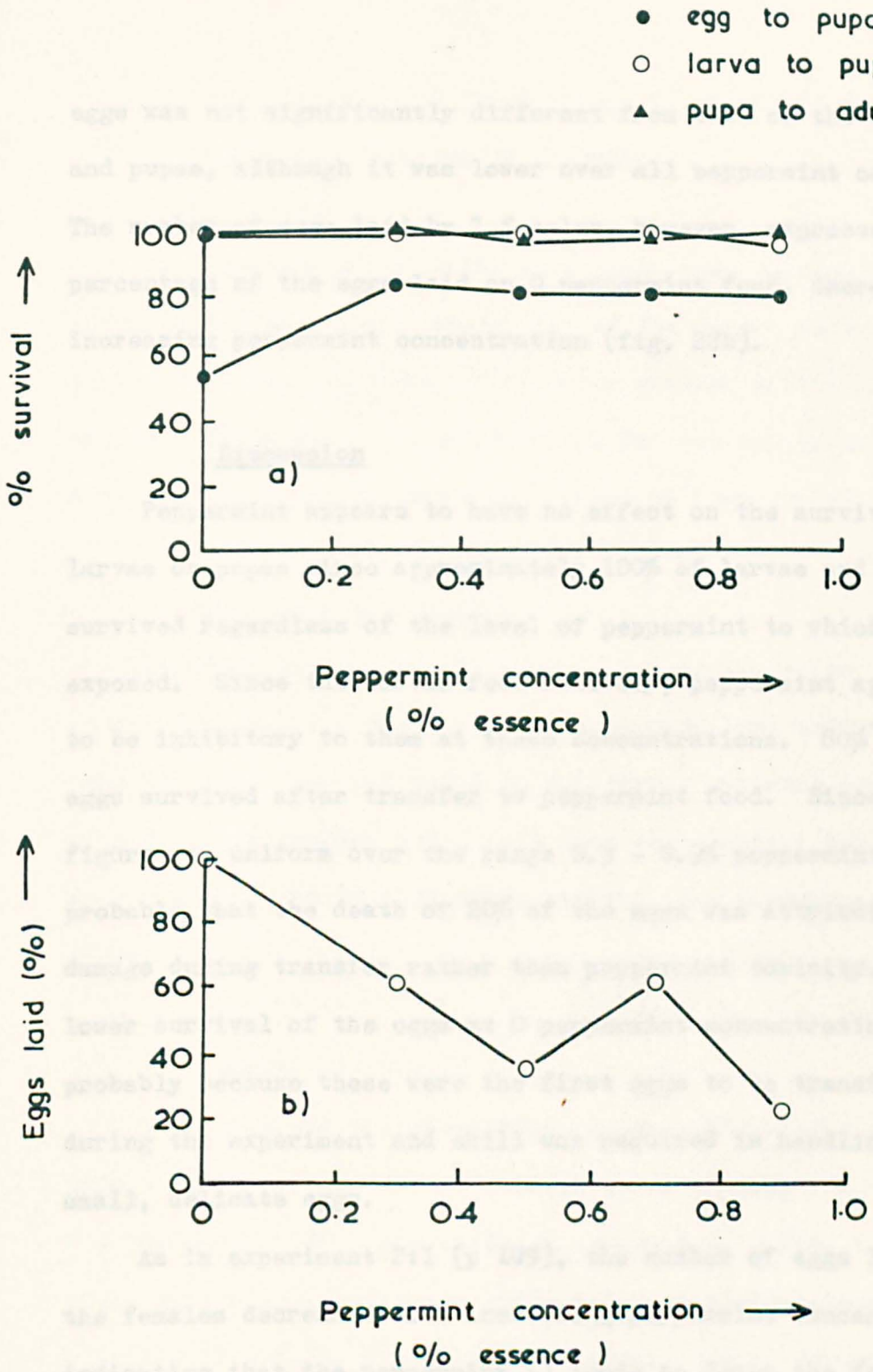


Fig. 22. a) The effect of peppermint on the survival of eggs, larvae and pupae.

b) The effect of peppermint on egg-laying (expressed as a % of the number of eggs laid by 7 females at 0 peppermint concentration).

(Standard errors in table 3, appendix 2, p 217).

eggs was not significantly different from that of the larvae and pupae, although it was lower over all peppermint concentrations. The number of eggs laid by 7 females, however, expressed as a percentage of the eggs laid on 0 peppermint food, decreased with increasing peppermint concentration (fig. 22b).

Discussion

Peppermint appears to have no effect on the survival of larvae or pupae since approximately 100% of larvae and pupae survived regardless of the level of peppermint to which they were exposed. Since the larvae feed actively, peppermint appears not to be inhibitory to them at these concentrations. 80% of the eggs survived after transfer to peppermint food. Since this figure was uniform over the range 0.3 - 0.9% peppermint, it is probable that the death of 20% of the eggs was attributable to damage during transfer rather than peppermint toxicity. The lower survival of the eggs at 0 peppermint concentration is probably because these were the first eggs to be transferred during the experiment and skill was required in handling the small, delicate eggs.

As in experiment 2:1 (p 109), the number of eggs laid by the females decreased with increasing peppermint concentration, indicating that the peppermint 1) tends to deter the females from egg-laying or 2) tends to prevent the flies from mating. In this experiment, there was no female mortality, but very high concentrations have been shown to be lethal to the adults. It is possible that at the lower concentrations used here, the adults suffer some distress. Perhaps both of the above factors may be involved in reducing the fecundity of the females.

2:3 Investigation of the Response of Different Families of D. melanogaster to peppermint

The previous experiments have shown that peppermint adversely affects the productivity of the flies, reducing fecundity of the females. In order to discover whether sufficient variability existed in the CB7 stock for the peppermint to elicit a selective response, individual families were tested.

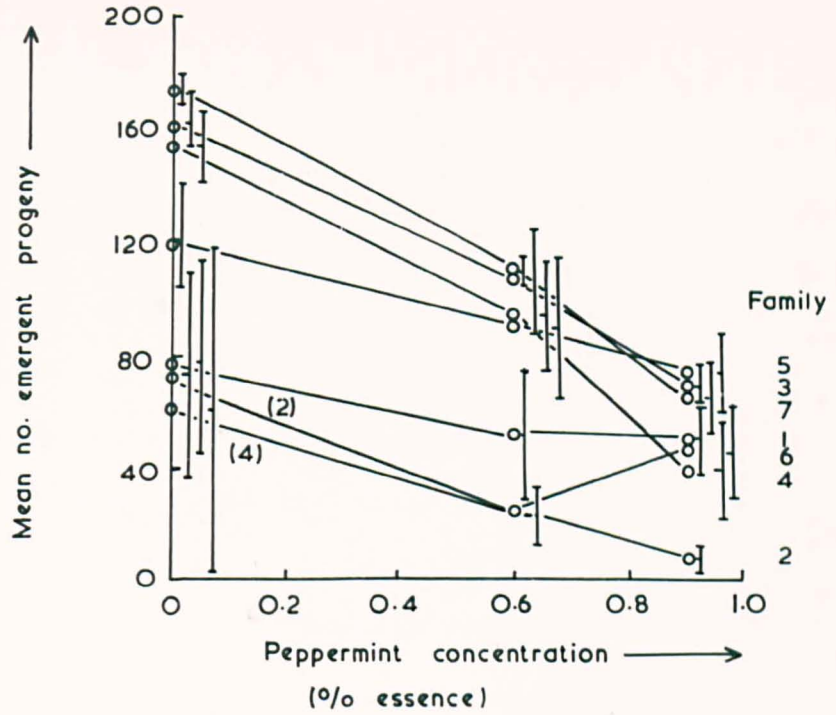
Materials and Methods

Eight individual families were bred in isolation from 1 virgin female and 1 male. Vials containing 10ml medium of three concentrations (0, 0.6 and 0.9% peppermint essence) were set up with 2 virgin females and 1 male from each family, replicated as many times as family size would permit. The progeny were counted on day 18.

Results

The different concentrations of peppermint had a significant effect on the yield of the families (from analysis of variance, $F = 18.94$, $P < 0.001$; see table 4, appendix 2, p 218; also fig. 23a). There were also significant differences in yield between the families ($F = 7.23$, $P < 0.01$). On normal food (0 peppermint concentration), these differences in yield indicated fertility differences between the families and residual effects of these were present at 0.6% peppermint concentration. They were not found at 0.9% peppermint concentration. The yield decreased with increasing peppermint concentration and it may be that, due to the unavoidably low number of replicates, (particularly in family 6), the smaller

a) expressed as number of emergent progeny



b) expressed as a proportion of the number of progeny at O peppermint concentration

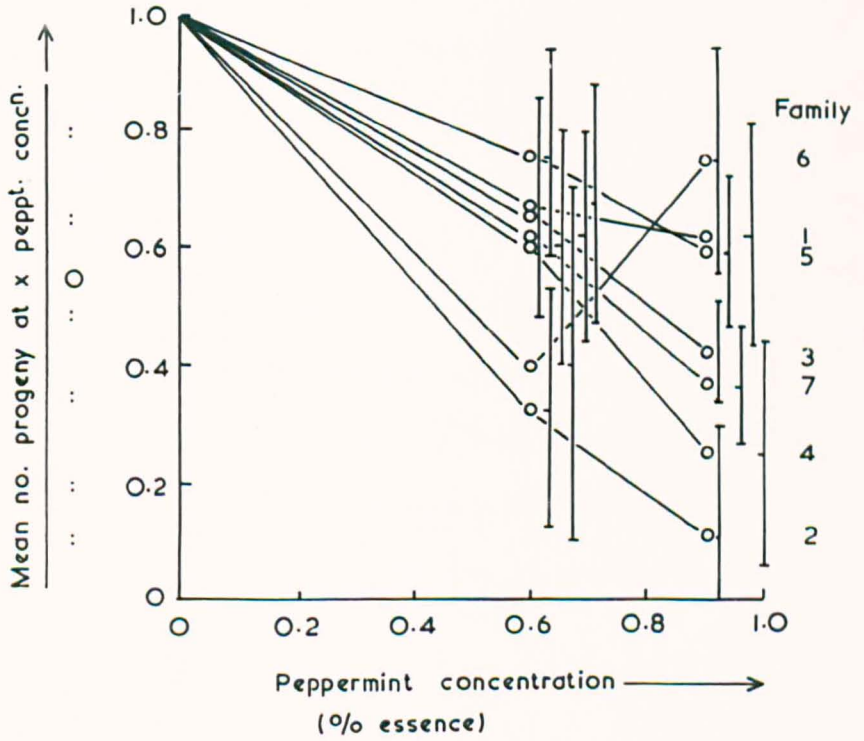


Fig. 23 Family response to peppermint.

yields at 0.9% peppermint concentration combined with the large error factor in the analysis to mask any fertility differences between the families (see discussion).

To separate the fertility differences from the response to peppermint, the number of emergent progeny of each family at each peppermint concentration was expressed as a proportion of the average number of emergent flies of that family at 0 peppermint concentration (fig. 23b). At 0.6% peppermint concentration, the families were not significantly different from each other in yield ($F = 0.770$). Again, the smaller proportions may have combined with the large error factor to mask underlying differences, which were indicated previously. At 0.9% peppermint concentration, however, significantly different family yields were found ($F = 9.55$, $P < 0.01$) and, therefore, the families differed in the extent of their response to peppermint at this concentration. For example, family 1 was less affected by the higher peppermint concentration, with a yield of 38% fewer progeny than on normal food. Family 2 yielded 89% fewer progeny on 0.9% peppermint food. These two families differed significantly in their response to peppermint but on normal food there were no fertility differences between them. Therefore, in addition to variability for fertility differences, there appears to be variability for response to peppermint within the CB7 stock.

Discussion

Significantly different family sizes were obtained on normal food, indicating fertility differences between the founder females (which were sibs). Therefore there was genetic variability within the CB7 *Drosophila* stock for this character. The significant

differences between families were also found on 0.6% peppermint food though not at the higher peppermint concentration. The extent of replication of the experiment depended on the sizes of the families produced by the sibs from one female. There were approximately 30 flies (males plus females) per family available for the experiment. As each replicate required 2 females and there were 3 treatments, there were few replicates. Also at 0.9% peppermint concentration, there were few flies produced in each family. Therefore, the statistical error at this concentration was comparatively greater than at the lower peppermint concentrations, which would mask underlying fertility differences.

Increasing peppermint concentrations effectively reduced family size. In general, the results confirm the conclusion of experiment 2:1, namely, the higher the peppermint concentration, the lower the yield. The largest families on normal food also tended to be the largest families on peppermint, e.g. family 1 was larger than family 2 at each peppermint concentration. However, when fertility differences were removed by expressing yield on peppermint as a proportion of that on normal food, family 1 yielded 38% fewer progeny compared with 89% fewer for family 2. These widely differing responses are significantly different and imply a genetic component.

Therefore, it has been demonstrated that, within the CB7 stock, there is genetic variability for fertility on normal food and for response to peppermint.

2:4 Summary of Preliminary Experiments

Peppermint in high concentrations (1.0% peppermint essence) was shown to be toxic to adult flies of the CB7 stock. At lower concentrations, it was not necessarily lethal but reduced the yield of the adults. Eggs, larvae and pupae appeared to be unaffected by peppermint when transferred to it from normal food. It was therefore suggested that lower yields on lower, less toxic concentrations of peppermint were brought about by the flies being deterred from either mating or egg-laying and not from adult mortality. It was demonstrated that, within the CB7 stock, there was some genetic variability for fertility and also for response to peppermint. As peppermint has been shown to reduce fertility, it would therefore provide a suitable selection agent for the main investigation. 0.5% peppermint food, together with normal food (0% peppermint), was chosen to provide the environmental heterogeneity necessary in the main experiment.

3. Study of Sympatric Divergence in a Laboratory Population of D. melanogaster

Materials and Methods

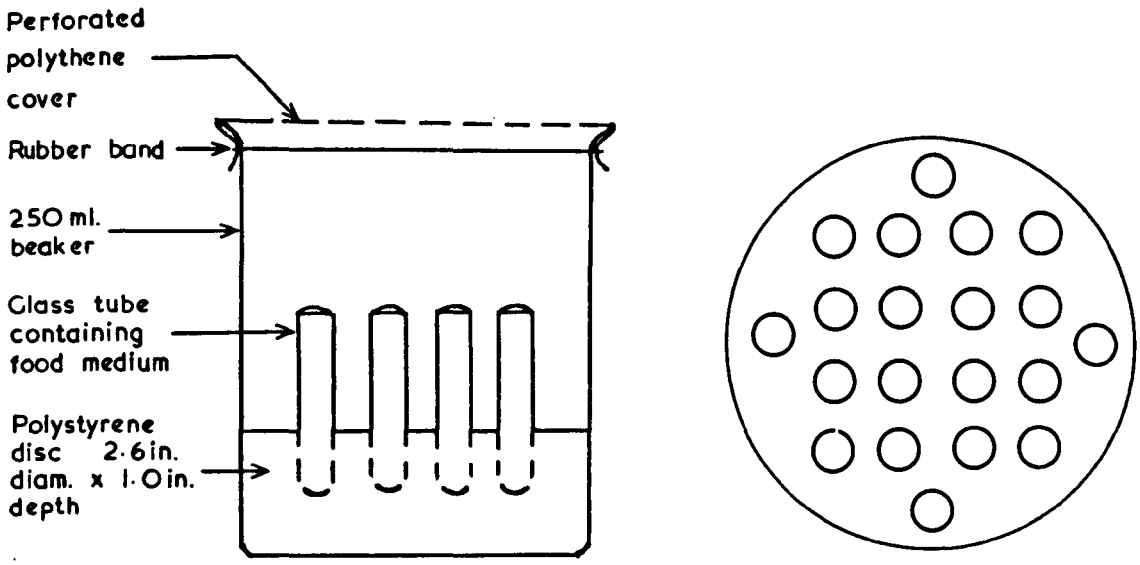
The main object of the investigation was to determine whether, within a small, randomly-mating population of D. melanogaster, sympatric divergence could be promoted by the heterogeneity of the environment.

The CB7 stock of flies was used for the experiment (see Appendix 2, p 213 for life cycle) as it had been shown to be genetically very variable (Antonovics, pers. comm.).

The heterogeneity to be experienced by the experimental population was provided by two alternative types of food medium. The food provides nourishment but is also the medium on which the eggs are laid and in which the larvae develop. It is therefore a major part of the environment of any Drosophila population. The two foods used to provide the heterogeneity were 1) normal food,(N),(medium described by Mittler & Bennett, 1962, with nipagin instead of propionic acid as a fungicide - see Appendix 2, p 212 for preparation), 2) this same food containing 0.5% peppermint essence, (P).

The 'population cages' in which the flies were kept, were 250ml glass beakers, containing 20 small glass tubes of food, standing in a polystyrene base, and sealed by a perforated polythene cover (fig. 24; see Appendix 2, p 219 for details of preparation).

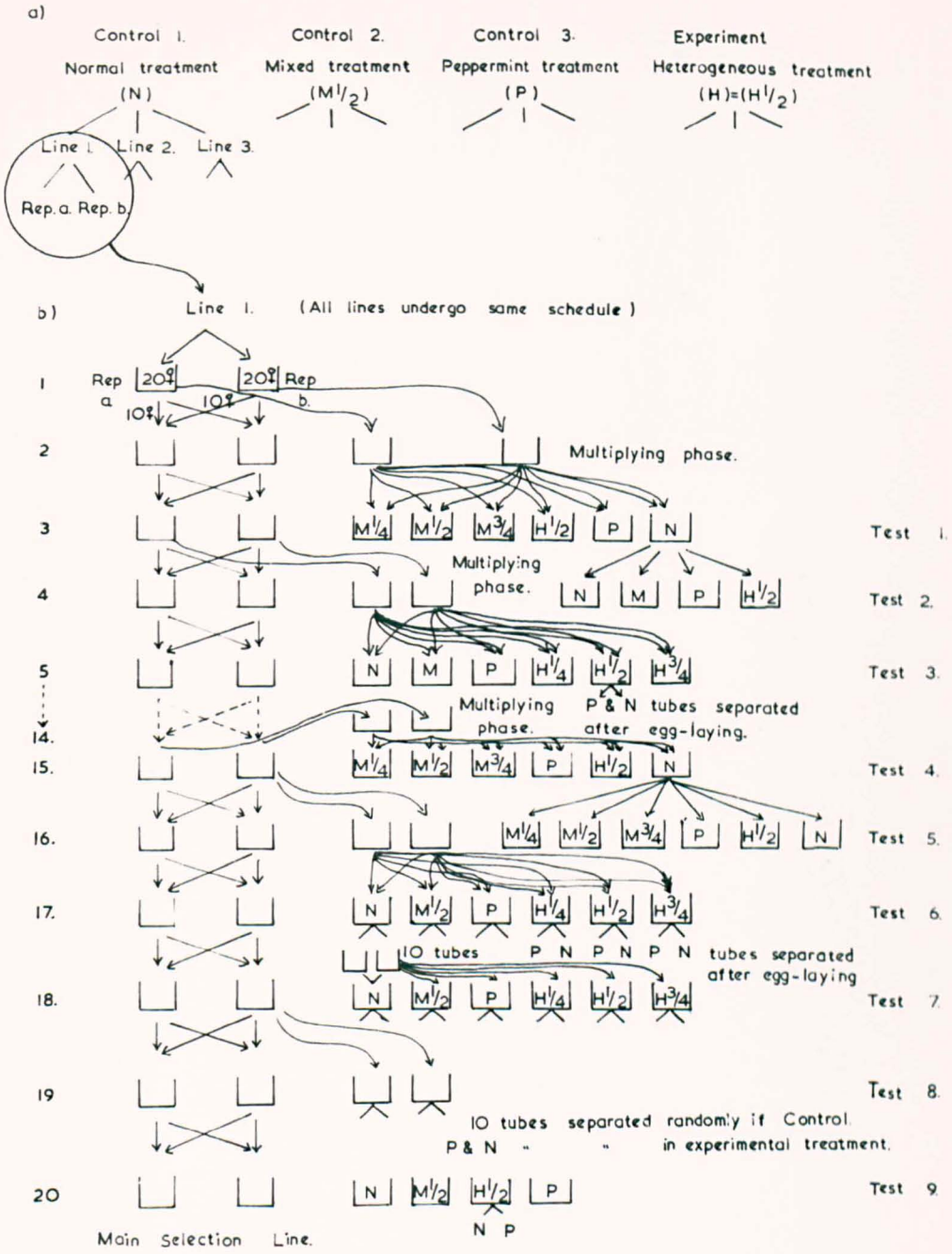
The experimental design is shown in figs. 25a and b. Four treatments, consisting of an experimental regime and three controls,



a) Lateral view of container

b) Position of tubes viewed from above

Fig. 24 'Population cage' used for main experiment.



Key to tests:

N - normal food

M 1/4 - 0.125 % peppermint essence
M 1/2 - 0.250% " "
M 3/4 - 0.375 % " "
P - 0.500% " "

H 1/4 - 5P: 15N tubes of food
H 1/2 - 10P: 10N - - "
H 3/4 - 15P: 5N " " "

Fig. 25 Experimental schedule to investigate the adaptation of D. melanogaster to a heterogeneous environment.

were created by the presence or absence of peppermint. In the experimental situation - the heterogeneous environment (H) - there were 10 tubes of normal food and 10 tubes of 0.5% peppermint food arranged at random within each beaker (see Appendix 2, figs. 1 and 2, pp 220 - 221 for randomised food arrangements). The 3 controls were as follows: 1) normal control (N) - 20 tubes per beaker of normal food; 2) peppermint control (P) - 20 tubes per beaker of 0.5% peppermint food; 3) homogeneous control (M) - 20 tubes per beaker of 0.25% peppermint food. The control (M) and the experimental regime (H) contained the same total amount of peppermint, but its distribution was homogeneous in the control (M) as against heterogeneous in the experimental situation (H). Each of the controls and the experimental regime comprised 3 replicate lines of flies, each line housed in two beakers, which were kept separate throughout the experiment, which lasted for 20 generations. The flies were allowed to mate at random within their own beaker. 20 females (10 per replicate beaker - to minimise inbreeding) were transferred at random after 21 days to fresh medium to form the next generation. The females were taken off after 6 days. Their progeny were counted on day 18 to measure the yield for each generation. After the first 2 generations, the flies reared on peppermint looked larger than those reared on normal food. Therefore, 150 flies per beaker were weighed together in order to obtain a reliable estimate of the weight of an average fly within the population. From this value, the biomass within each beaker was calculated for each generation.

After the first 2 generations, the genetic and environmental response of the flies from the experimental regime (H) and the

3 controls N, M and P, was assessed by measuring the population size as follows, the main selection lines being kept distinct from the test situations:

Test 1. Yield (number of emergent progeny) at different homogeneous peppermint concentrations of 0, 0.125, 0.250, 0.375 and 0.500% essence to test the initial response to peppermint.

Test 2. Yield (number of emergent progeny) on different homogeneous peppermint concentrations after being on normal food for one generation, to test for the extent of selection, if any.

For tests 1 and 2, population size was measured as number of emergent progeny but for the subsequent tests, biomass was also obtained.

Test 3. Yield (number of emergent progeny and biomass) at different heterogeneity levels (in which the total amounts of peppermint per container were similar to those in test 1 but were distributed heterogeneously) to test for the initial response of the flies to the heterogeneous situation. (For the arrangement of tubes at the $\frac{1}{4}$ and $\frac{3}{4}$ heterogeneity levels see Appendix 2, fig. 2, p 221).

In test 3, the normal and peppermint tubes in the heterogeneous situations were separated after egg-laying and placed in fresh containers to compare egg distribution on the two media and to separate the progeny for counting and weighing. By mistake, normal and peppermint tubes in the main heterogeneous lines (as well as in the test populations) were also separated at generation

5 and this caused an anomaly (see results p 127). Since the separated flies were then at a different density from those in the control situations, the results were not comparable. In later tests (6 and 7), this was overcome by also separating 10 tubes at random from each of the control containers and putting them into fresh containers. After the separation of the tubes in test 3, dummy tubes, containing no food, were used to bring the total number of tubes in the fresh container back to 20. This was done because the tubes provided pupation sites and it was thought that the yield might be affected if the number of pupation sites was reduced by half. However, subsequent experimentation revealed that this was not the case and that it was not necessary to use dummy tubes.

The lines were maintained until generation 15, when the flies were again assessed for genetic and environmental response in a series of tests similar to those at the beginning of the experiment:

Test 4. As test 1 - to assess peppermint tolerance after 15 generations.

Test 5. As test 2 - to test whether any response to peppermint after 15 generations is a genetic response.

Test 6. As test 3 - to test response to heterogeneity - after 17 generations.

Test 7. As test 3 after 1 generation of lapsed selection to test whether any response to heterogeneity is residual and probably, therefore, genetic.

(Tests 6 and 7 were modified in design from test 3, as previously recorded).

The lines were then tested for egg-laying response and the

development of habitat-choice preferences, which might have arisen during the experiment.

Test 8. To investigate the number of eggs laid by females from the experimental regime (H) and the 3 controls (N, M and P) after 19 generations of selection.

Test 9. To investigate the possible existence of habitat-choice preferences in the females after 20 generations of selection.

Results

The results of this experiment to investigate sympatric divergence within a small population in a heterogeneous environment fall into 4 sections:

i) The population size, measured for 18 generations, of the experimental population (H) and the three control populations (N, M and P), indicating their adaptation to the experimental system in general and providing a comparison between them.

ii) The results of the tests for adaptation to peppermint (tests 1, 2, 4 and 5).

iii) The results of the tests for adaptation to heterogeneity (tests 3, 6 and 7).

iv) The results of the tests of habitat selection (tests 8 and 9).

i) Population size

a) Number of emergent flies

There was an overall increase in the number of emergent flies in the experimental population (H) and in the three controls (N, M and P), (fig. 26a; Appendix 2, table 5, p 222). In the heterogeneous population (H) after egg-laying at generation 5, tubes containing normal food were separated from tubes containing peppermint food and placed in fresh containers by mistake. The total number of emergent progeny obtained from the two beakers (>400) was, therefore, not comparable with that obtained for the three controls, since the flies were at a different density. For this reason, the data at this point were not used in the statistical analysis that follows.

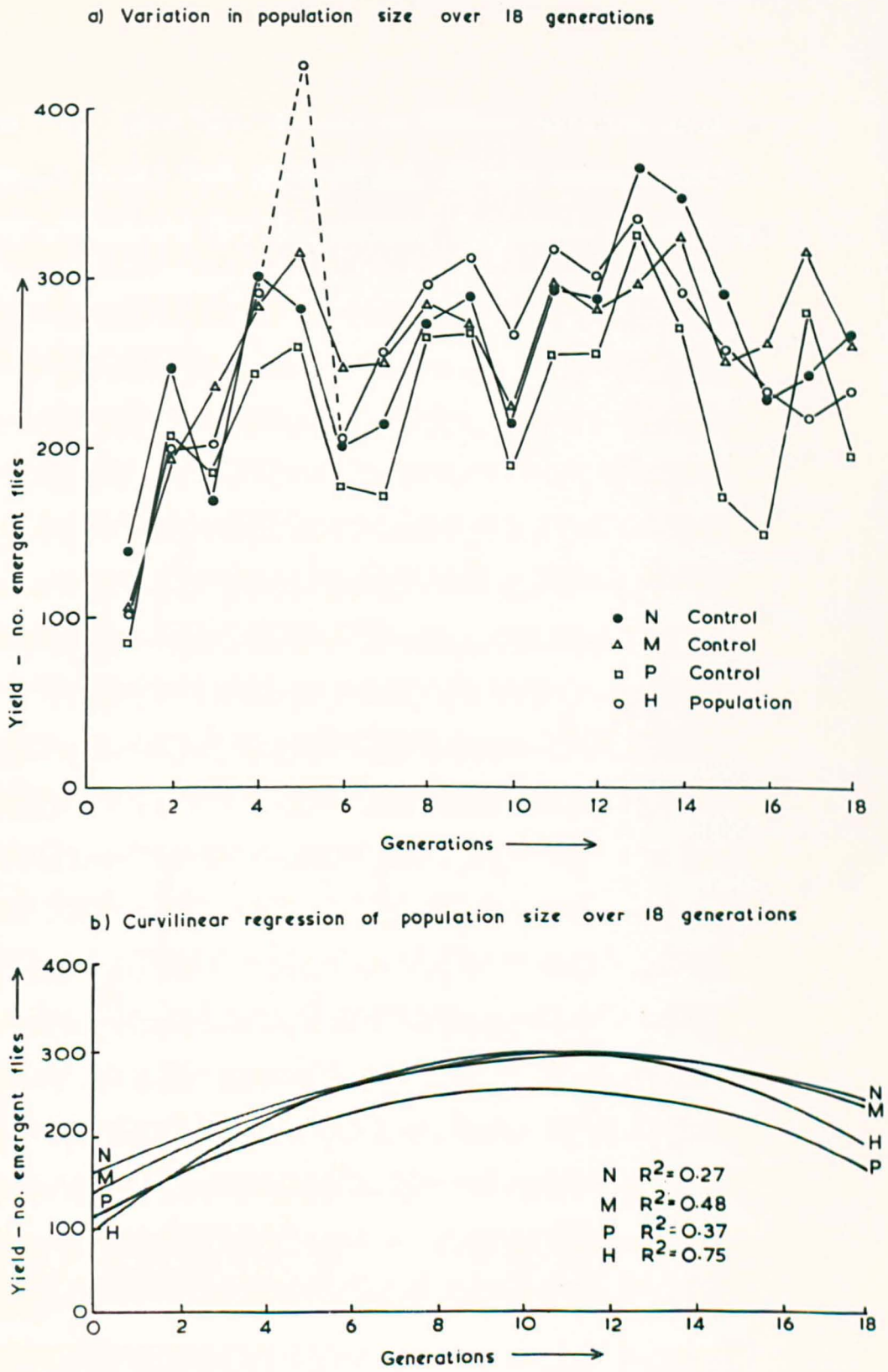


Fig. 26 Population size over 18 generations.

(Standard errors in table 5, appendix 2, p 222.)

There were large fluctuations in numbers from one generation to the next in all four populations. It did appear, however, that the M control and the experimental population (H) were not subject to such large fluctuations as were the N and P controls.

In order to show the underlying trends, a curve was fitted through each set of data by curvilinear regression (fig. 26b). Because of the fluctuations noted above, the R^2 values, which indicate the extent to which the data fit the line, are generally low (fig. 26b). However, 75% of the data for the heterogeneous population (H) fits the curve compared with 25 - 50% for the three controls. Of the four populations, therefore, the heterogeneous population is less subject to fluctuations.

The curves also show that the numbers of progeny in all four populations, rose during the experiment to reach an optimum at generation 11. This level was approximately 300 flies for the N and M controls and the experimental population (H) and 250 for the P control. These numbers were not significantly different from each other at generation 11 (analysis of variance: F = 2.41, NS), but the numbers of progeny in the P control were consistently lower throughout the experiment. The increase in numbers from generation 1 to generation 11 was, however, significant in all cases (table 18).

Table 18. Results of t-test on number of emergent flies

Population	t^1	sign.	t^2	sign.
N	4.17	0.05	2.46	N.S.
M	6.40	0.05	1.55	N.S.
P	10.55	0.01	0.68	N.S.
H	5.58	0.05	5.01	0.05

t^1: test between generations 1 and 11,
t^2: " " " 1 and 18.

After generation 11, the number of progeny decreased in all four populations and was still decreasing at the close of the experiment. The yield at that time was still higher than during the early generations but, except for the experimental population (H), not significantly so (table 18).

Comparison of the observed and expected population size (yield) of the experimental population (H) in fig. 27a shows that for the first 12 generations there was a rapid build-up of excess flies in the H population, which was then lost as the numbers in all populations decreased. Greater than expected numbers of progeny were observed in the H population for the most generations, but, regressed over all the generations, this was not significant. The curves found by curvilinear regression (fig. 27c) show these results more clearly. The H population and the M control were not significantly different from each other (fig. 27b) but the yield of the H population was generally greater than that of the M control.

b) Average weight per fly

The average weight per fly was obtained for each generation (except generation 12, when there were technical difficulties) for each population (fig. 28a; Appendix 2, table 6, p 223). The four populations were not significantly different from each other. There were fluctuations in weight from one generation to the next but the underlying trends are revealed by curvilinear regressions (fig. 28b). Flies reared on peppermint (M and P controls) were found to be initially heavier in weight than those reared on normal food (N control). Flies reared in the heterogeneous environment were initially intermediate in weight between the two.

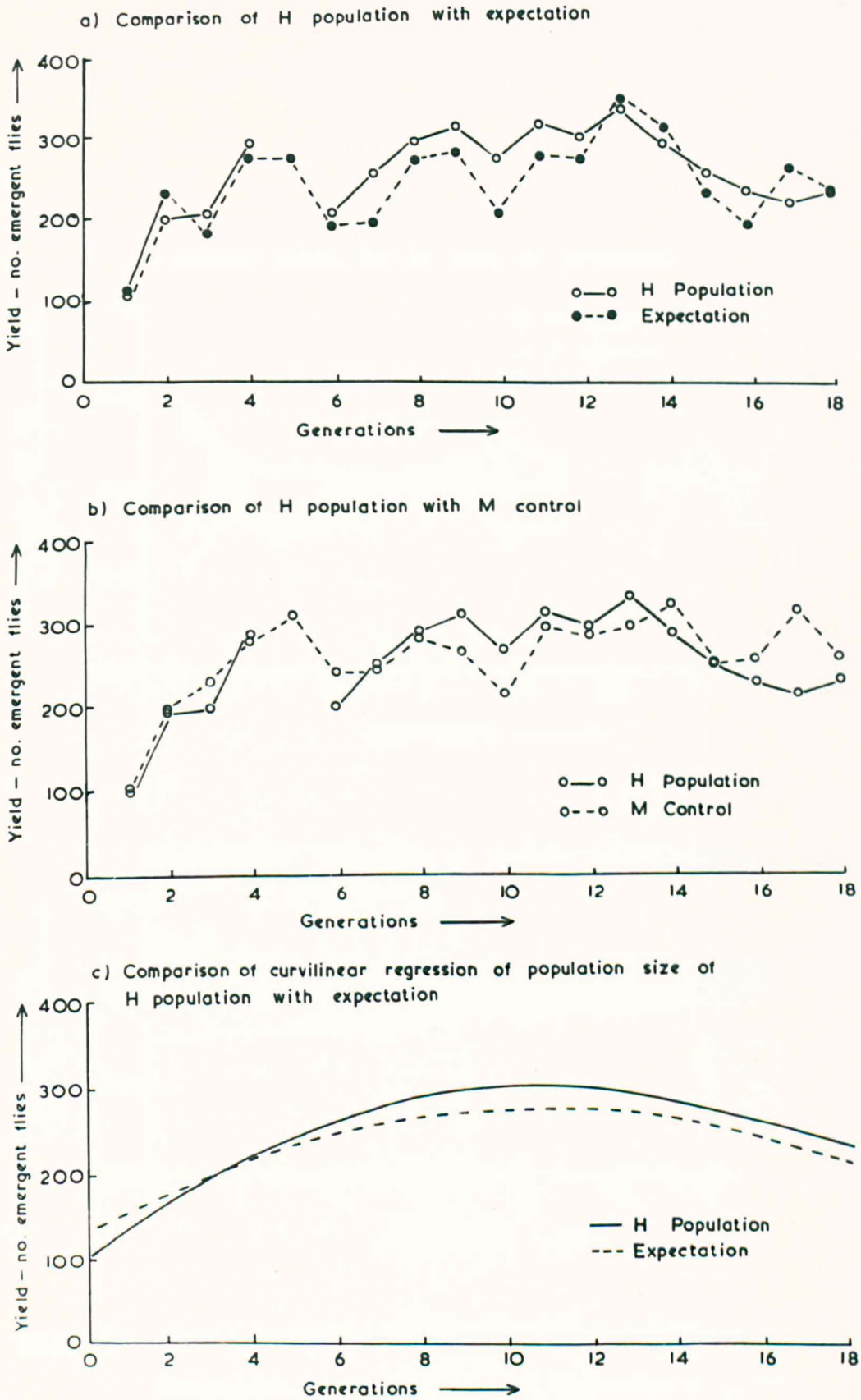


Fig 27 Comparison between H population and (a) expectation (b) M control. (Standard errors in table 5, appendix 2, p 222.)

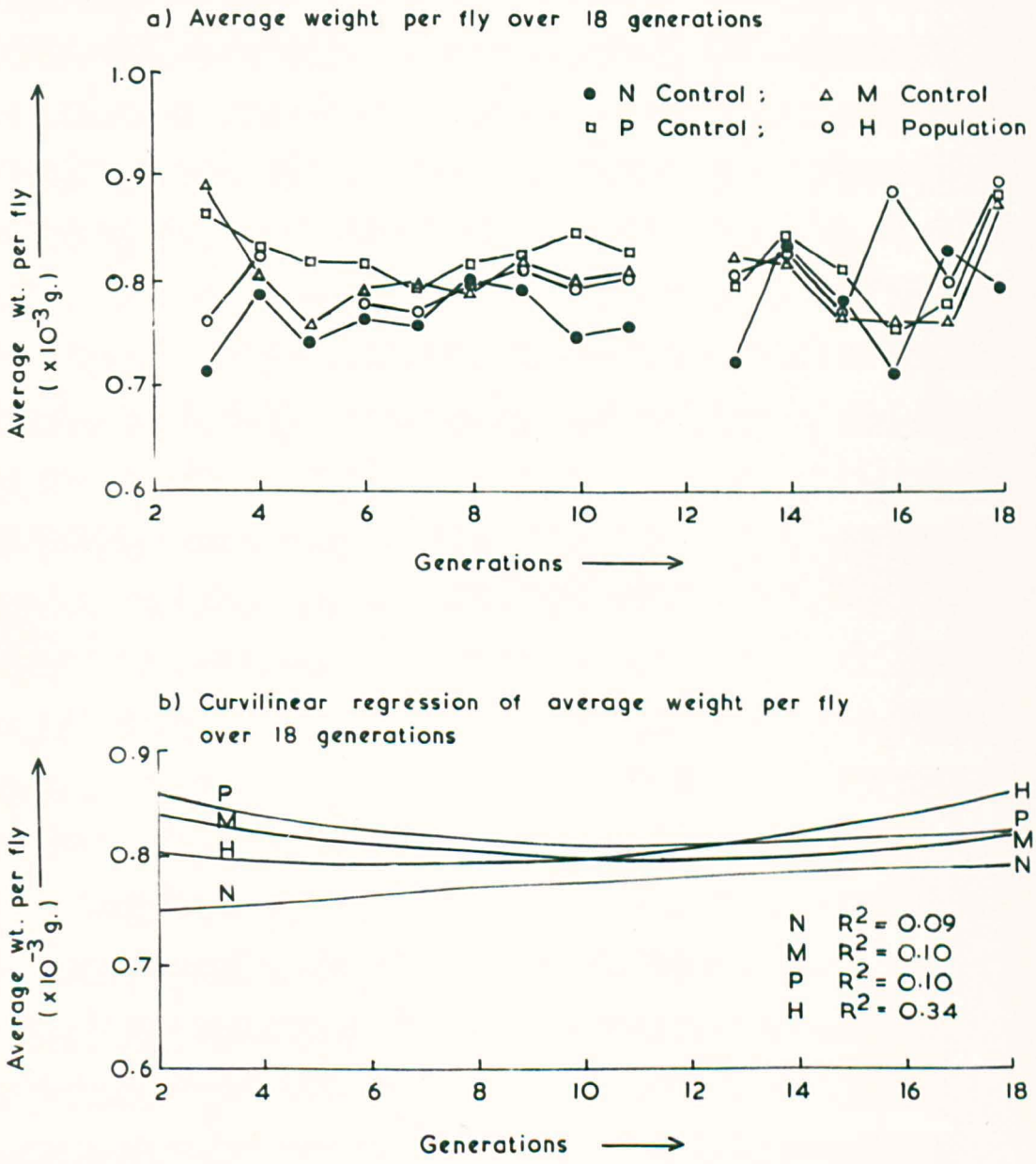


Fig. 28 Average weight per fly over 18 generations.

(Standard errors in table 6, appendix 2, p 223.)

The N control flies became heavier in weight throughout the experiment, although never attaining the weight of the flies in the other controls or experimental population. Flies in the P control decreased in weight until generation 11 after which their weight remained more or less constant. Flies in the M control decreased in weight until generation 11, after which they increased in weight, though never achieving their initial weight. Flies in the H population remained intermediate in weight between the N and P control (not significantly different from expectation) until generation 9. They then began to increase in weight, becoming heavier on average than any of the other flies from generation 13 till the end of the experiment.

It was found by analysis from a subsequent test (test 4; Appendix 2, tables 10a & b, p 227) that the average weight of a fly is highly correlated with the density of its parental population ($r = -0.75$, $P < 0.001$), i.e. the larger the average weight per fly, the smaller the population from which it came, regardless of the food medium (see fig. 29). Because of this and other density effects (see discussion, p 160), the populations all show large fluctuations in numbers, which are not always synchronised, from one generation to the next. Therefore, biomass (average weight per fly x number of emergent flies) represents a more 'reliable' measurement on which to judge the effect of the experimental treatment on the flies.

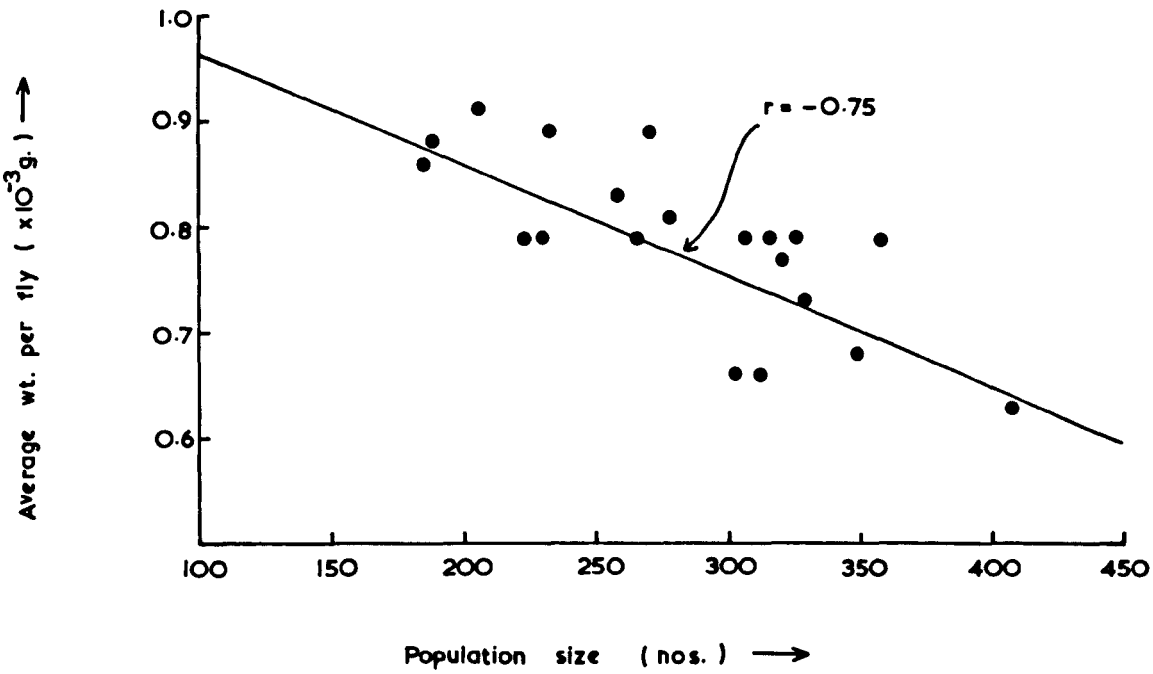


Fig 29 Relationship of average weight per fly with population size.

c) Biomass

When population size was expressed as biomass (total weight of emergent flies), it was found that, despite large fluctuations, there appeared to be a slight increase throughout the experiment for the heterogeneous population and the three controls (fig. 30a; Appendix 2, table 7, p 224). Curvilinear regressions for each population gave a clearer picture of the results (fig. 30b). It was discovered, however, that the increase in biomass was linear and that curvilinear regression did not describe the data more accurately than linear regression. The data were therefore analysed by linear regression, since tests of significance are available. The increase in biomass was found to be significant for the N control ($r = 0.63$, $P<0.01$) and the H population ($r = 0.50$, $P<0.05$), but not significant for the P and M controls. Independent t-tests, comparing the biomass of generations 3 and 18 for all populations, confirmed this result: for N control, $t = 4.34$, $P<0.05$; for H population, $t = 4.88$, $P<0.05$; for M control, $t = 2.40$, N.S. and for P control, $t = 1.79$, N.S.. Comparing the biomass of the 3 controls and the experimental population (H) throughout the experiment, P control population was generally the lowest and the M control population was initially the heaviest, although there were no significant differences between the populations. Each of the populations showed large fluctuations from one generation to the next. This effect was least marked in the heterogeneous population.

At generation 3, the biomass of the heterogeneous population was above expectation and the difference between the observed and expected biomass increased rapidly (fig. 31a). After generation 13, however, the biomass decreased but was generally in excess of

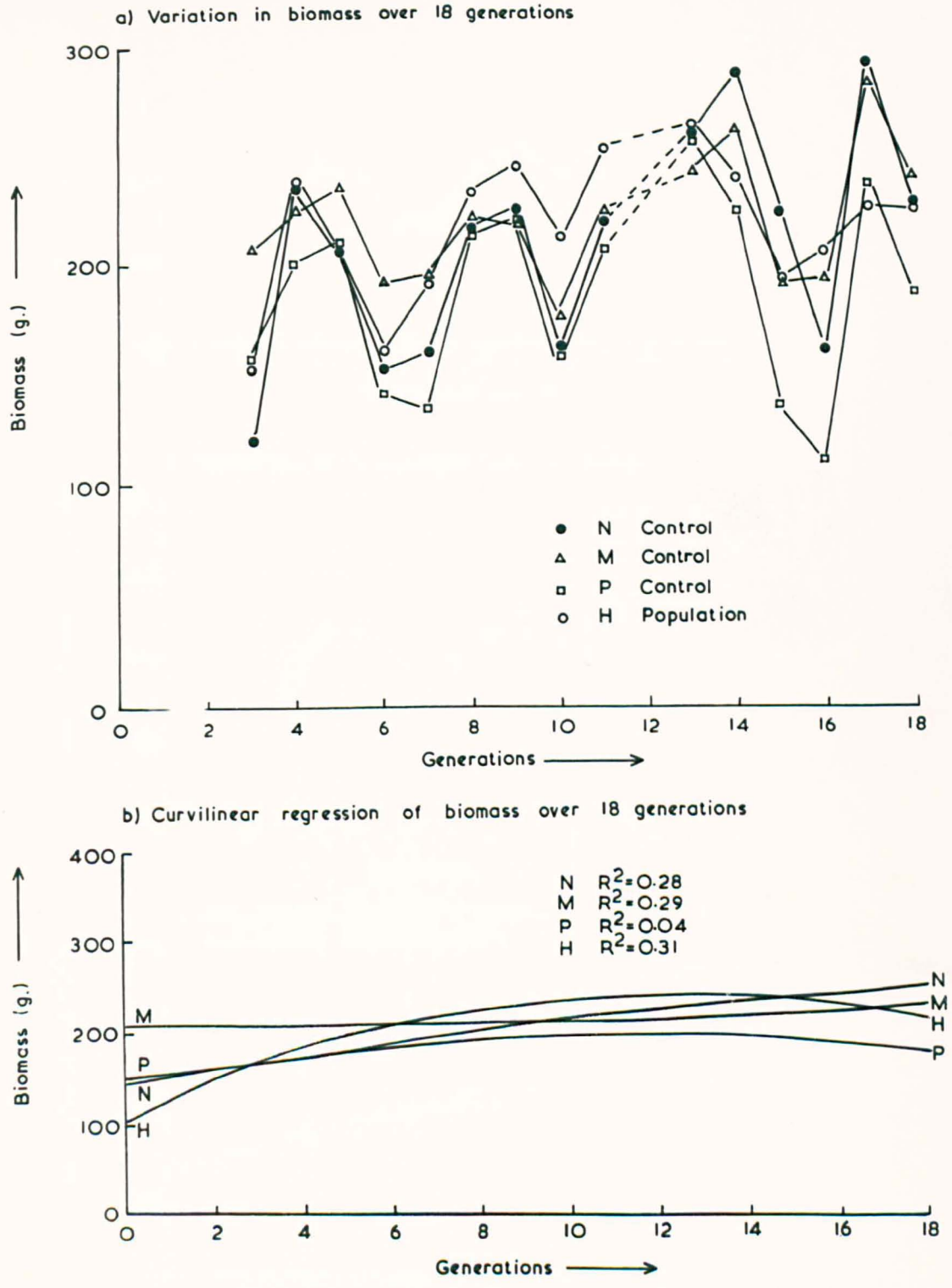


Fig. 30 Biomass over 18 generations.

(Standard errors in table 7, appendix 2, p 224.)

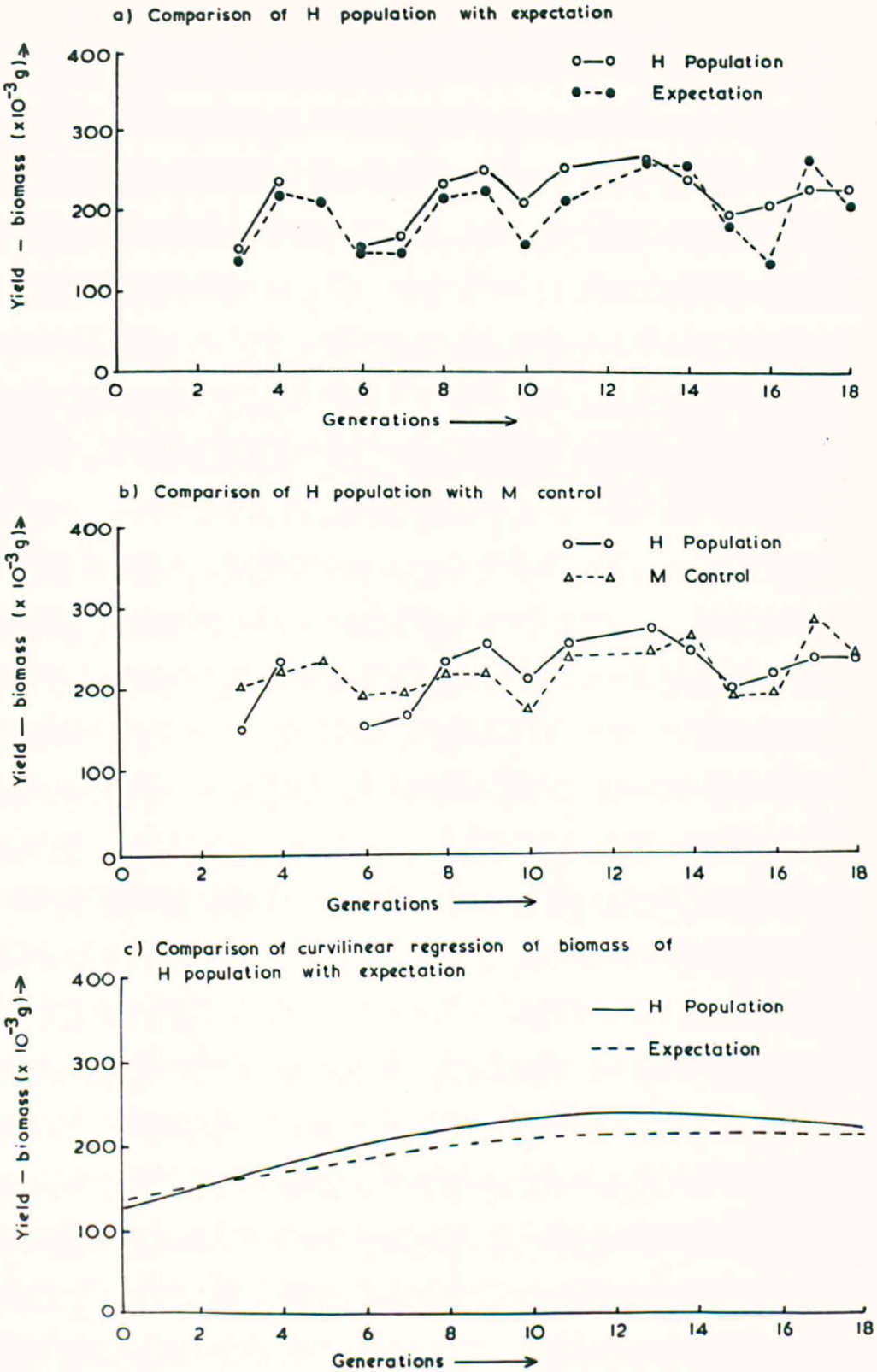


Fig. 31 Comparison between the biomass of the H population and (a) expectation, (b) biomass of the M control. (Standard errors in table 7, appendix 2, p 224.)

expectation. The biomass of the M control was higher than that of the heterogeneous population until generation 7 (fig. 31b). The biomass of the heterogeneous population then exceeded that of the M control until generation 14. There were, however, no significant differences in biomass between the two populations over 18 generations.

Summary

As commonly found in Drosophila experiments, there were large fluctuations in the numbers of emergent progeny in each population from one generation to the next. All four populations showed a rapid increase in numbers and, in the heterogeneous population, the numbers exceeded expectation, the difference between the observed and expected numbers gradually increasing until generation 12. Because the number of emergent flies is, to some extent, dependent on density effects within the population, it was important that these should be minimised so that the populations could be compared with respect to the variables in the heterogeneous environment, the foods. When the biomass of each population throughout the experiment was compared, (1) the flies reared on normal food showed increased adaptation to that food by a steady, significant increase in biomass; (2) the flies reared on peppermint (either 0.25% or 0.50% essence) showed no significant increase in biomass and, therefore, no adaptation to peppermint; (3) the heterogeneous population increased significantly in biomass and, for the greater part of the experiment, the biomass exceeded expectation. In particular, the difference between the observed biomass and expectation increased steadily from generation 3, when first measured, until generation 12.

ii) Tests for adaptation to peppermint

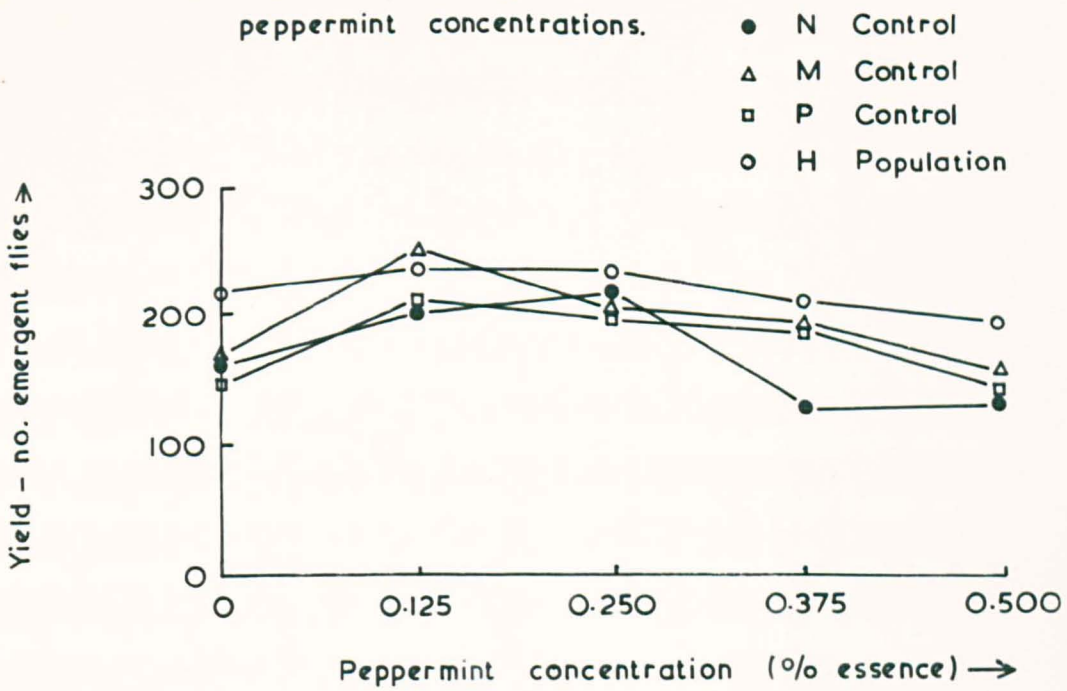
Tests 1, 2, 4 and 5, were designed to reveal to what extent any apparent improvement in population fitness (shown by increased population size) was genetic or a purely environmental response. The detailed results are presented in Appendix 2, tables 8, 9, 10a & b, 11a & b, pp 225 - 230. Measurements of biomass were not obtained for tests 1 and 2.

a) Test 1 - Yield on peppermint after 2 generations

Tests were carried out on a range of 0%, 0.125%, 0.250%, 0.375% and 0.500% peppermint essence. The four populations (N, M, P and H) showed an overall decrease in numbers of emergent progeny with increasing peppermint concentration (fig. 32a). In all four populations, fewer progeny were obtained on normal food than on 0.125% peppermint food.

Flies from the P control population did not yield more progeny than N control flies on peppermint food (0.500% essence). Hence there was no adaptation to the peppermint at this stage. The population size of the heterogeneous population (H) was generally greater over all peppermint concentrations. When the results are expressed as % reduction from the performance on normal food (fig. 32b), this population was the least affected by the different amounts of peppermint. The flies reared on normal food for 2 generations (N control) were less productive on peppermint than the flies reared on peppermint (P control), though not significantly so.

a) Yield on a range of peppermint concentrations.



b) Yield expressed as % increment from yield on 0 peppermint food (normal food).

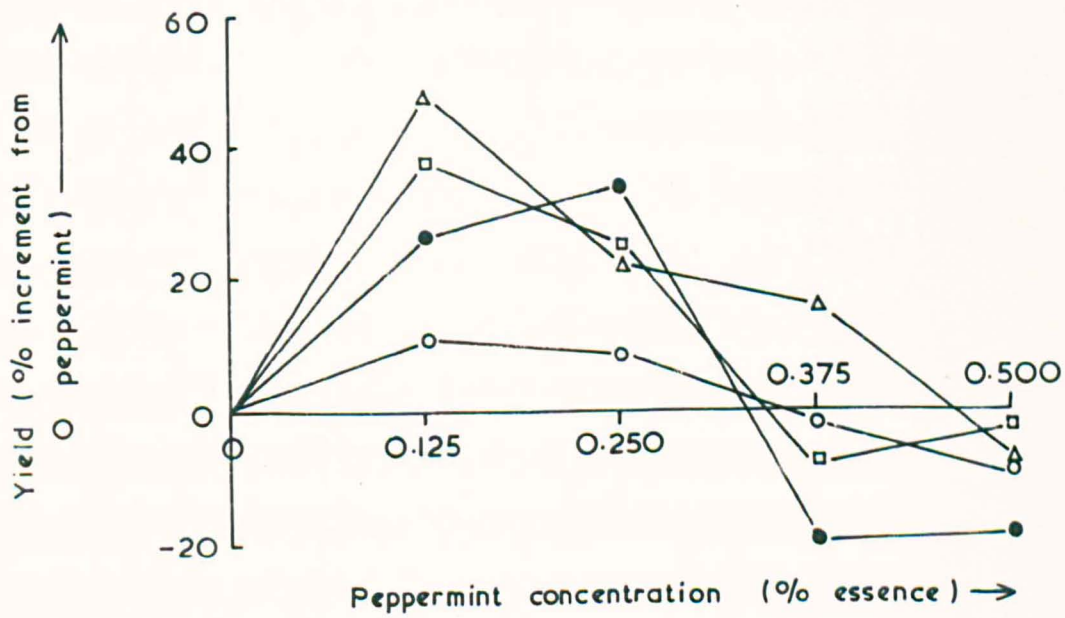


Fig. 32 Results of test 1 on a range of peppermint concentrations. (Standard errors in table 8, appendix 2, p 225.)

b) Test 2 - Yield on peppermint after 1 generation of lapsed selection on normal medium

There was no evidence of any genetic response to peppermint being built up over the first 3 generations. Figs. 33a & b show an overall decrease in numbers of emergent progeny with increasing peppermint concentration for each of the 3 controls and the heterogeneous population. Comparing these 4 populations after 1 generation of lapsed selection on normal food, flies from the P control population produced less offspring on peppermint than did flies from any other population. Therefore there appeared to be no response to peppermint at this stage of the experiment.

c) Test 4 - Yield on peppermint after 15 generations

There was no evidence of any genetic response to peppermint after 15 generations of selection. Fig. 34a shows that the numbers of emergent flies in the 3 control populations and the heterogeneous population decreased as the amount of peppermint increased. The biomass of the 4 populations (fig. 34b) shows little change with increasing peppermint concentration. Population size (both in biomass and numbers) of the flies in the heterogeneous environment was the least affected by different concentrations of peppermint. Flies of the M control produced many more flies than those of other controls over the intermediate range of peppermint concentration, indicating a possible response to selection in this control.

Comparison with test 1 (fig. 35) reveals that greater numbers of progeny were being produced by all the populations after 15 generations of selection, particularly on normal food (0 peppermint) and on low concentrations of peppermint. After 15 generations, however, there was little change in the numbers produced on 0.5%

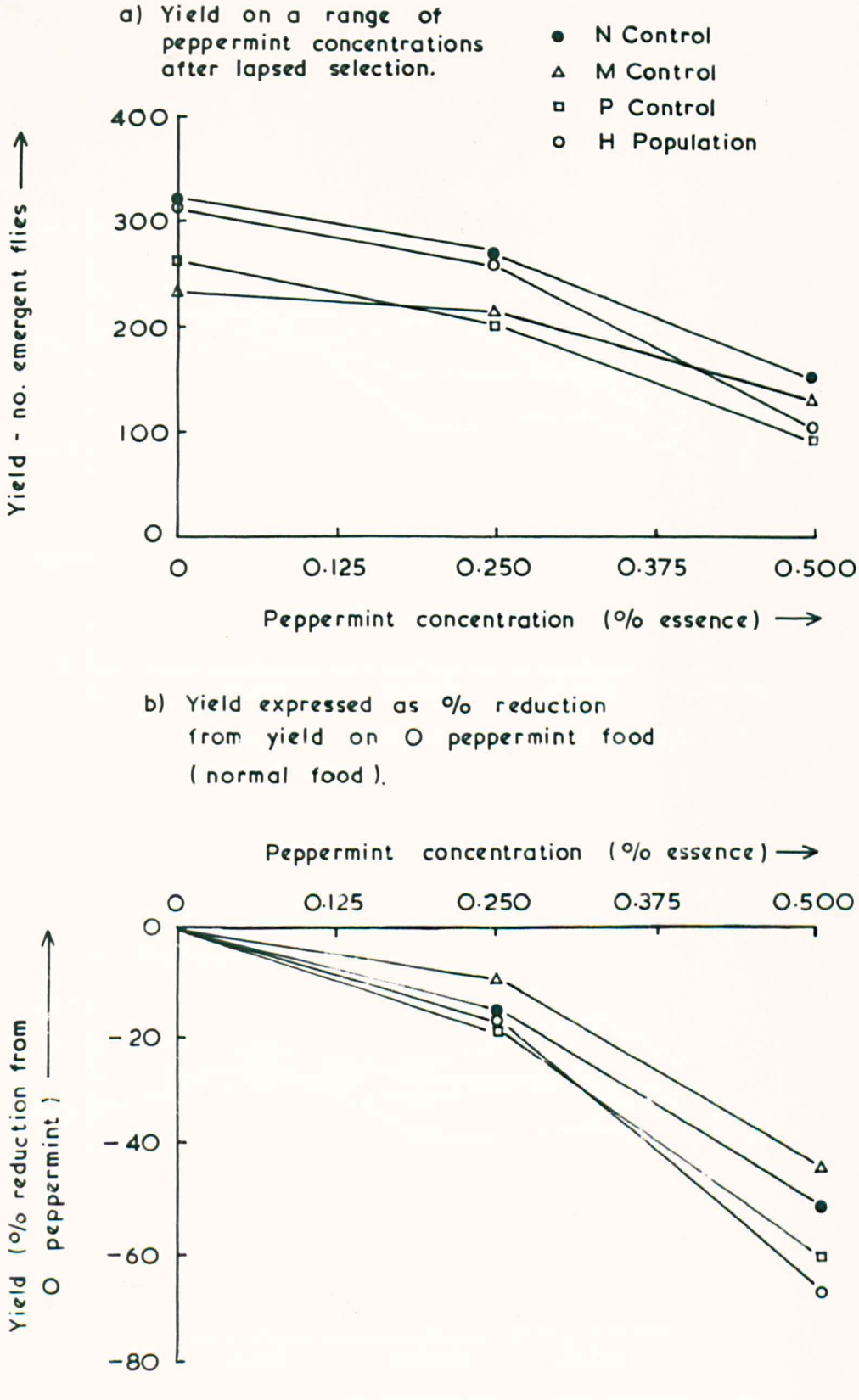


Fig. 33 Results of test 2 - yield on a range of peppermint concentrations after 1 generation of lapsed selection. (Standard errors in table 9, appendix 2, p 226.)

Yield on a range of peppermint concentrations after 15 generations of selection.

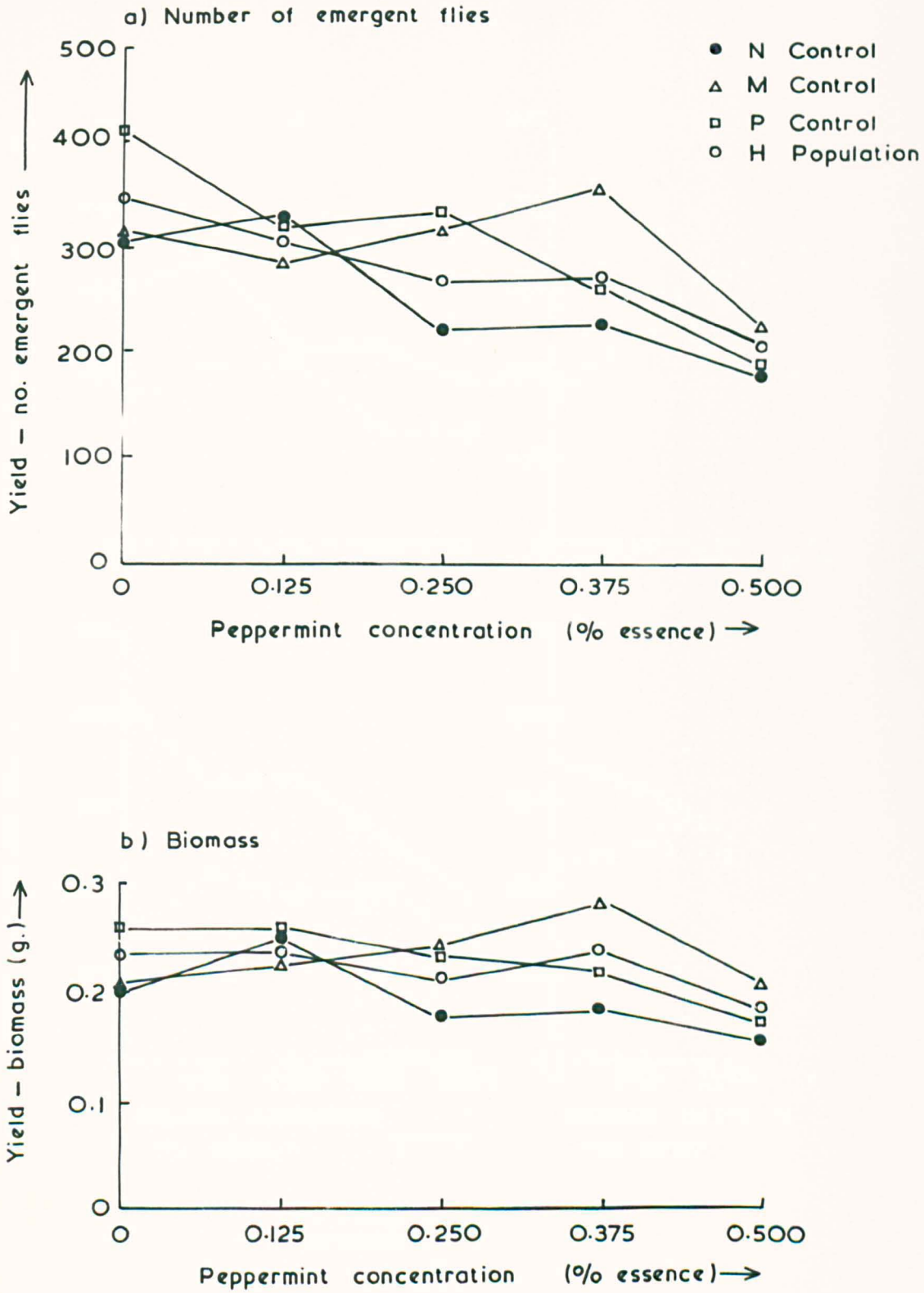


Fig. 34 Results of test 4 - yield on a range of peppermint concentrations after 15 generations of selection. (Standard errors in tables 10a & b, appendix 2, pp 227, 228.)

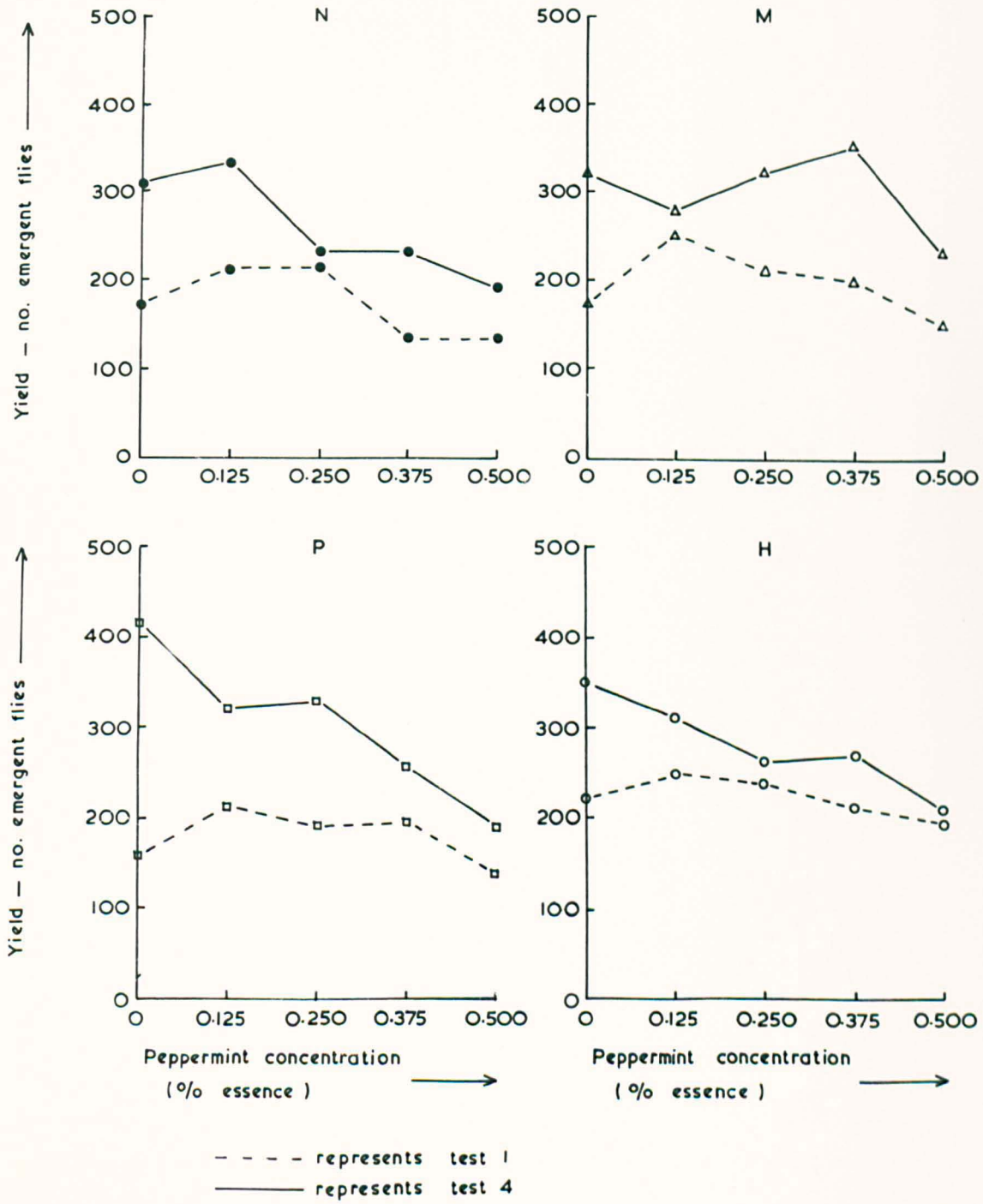


Fig. 35 Comparison of tests 1 and 4.

peppermint and there was no difference between the N and P control populations at this concentration.

d) Test 5 - Yield on peppermint after 15 generations of selection and 1 generation of lapsed selection on normal medium

The trend of decreasing population size (numbers and biomass) with increasing peppermint concentration was again observed in all 4 populations (fig. 36). The superiority of the M control over the intermediate range of peppermint concentrations was lost. Flies reared on peppermint (P control) produced fewer progeny on peppermint than those reared on normal medium (N control).

Summary

Comparison of the results of the early tests (1 & 2) and the later tests (4 & 5) shows that no measurable genetic adaptation to peppermint occurred over 15 generations of selection in populations reared on peppermint medium. There was an increase in the yield produced by the 3 controls and the heterogeneous population at each peppermint concentration. An improvement in the response of the M control population to low concentrations of peppermint was observed but this effect was lost after one generation on normal medium, suggesting an environmental rather than a genetic response.

Yield on a range of peppermint concentrations after 15 generations of selection and 1 generation of lapsed selection.

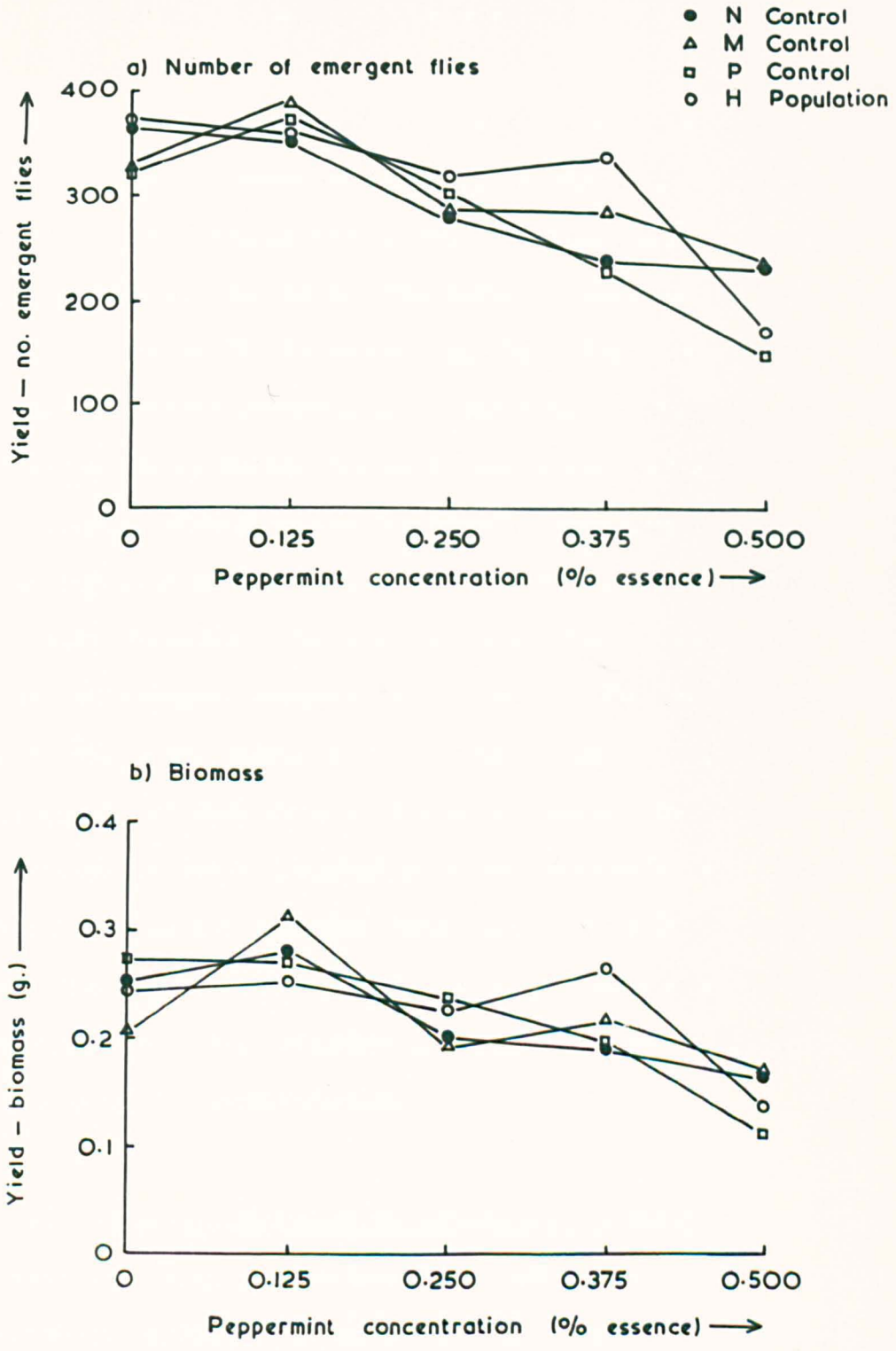


Fig. 36 Results of test 5 - yield on peppermint after 15 generations of selection and 1 generation of lapsed selection on normal medium. (Standard errors in tables 11a & b, appendix 2, pp 229, 230.)

iii) Tests for adaptation to heterogeneity

Test 3 (on generation 5) and tests 6 and 7 (on generations 17 and 18), were designed to discover whether adaptation to heterogeneity had arisen during the experiment and whether any response might be heritable. The detailed results are given in Appendix 2, tables 12, 13 and 14, pp 231 - 235. The results of each test are shown graphically in the text in the form of Replacement Series Graphs for each population (after De Wit 1960). This analysis, normally used for competition experiments, was used to indicate to what extent the two foods in the heterogeneous environment contributed to the resultant size of each population (numbers of emergent progeny and biomass). The numbers (or biomass) emerging from each food,and their totals,were plotted against the frequency of each food in the environment. The graphs were also plotted as ratio diagrams on a log-log scale, the log of the ratio of the numbers emerging from each food (P/N) being plotted against the log of the ratio of the food frequencies (P/N) within the environment. See Appendix 2, p 236, for some theoretical graphs and their interpretation.

a) Test 3 - Response to heterogeneity before selection

When tested in the heterogeneous environment with the 2 foods available in different proportions, (15N : 5P ($H\frac{1}{4}$), 10N : 10P ($H\frac{1}{2}$), 5N : 15P ($H\frac{3}{4}$)), the 3 control populations,(N, M and P), and the heterogeneous population (H) yielded greater-than-expected numbers of progeny (fig. 37). Biomass was not recorded for this test. The peak productivity at $H\frac{1}{2}$, i.e. when equal amounts of normal

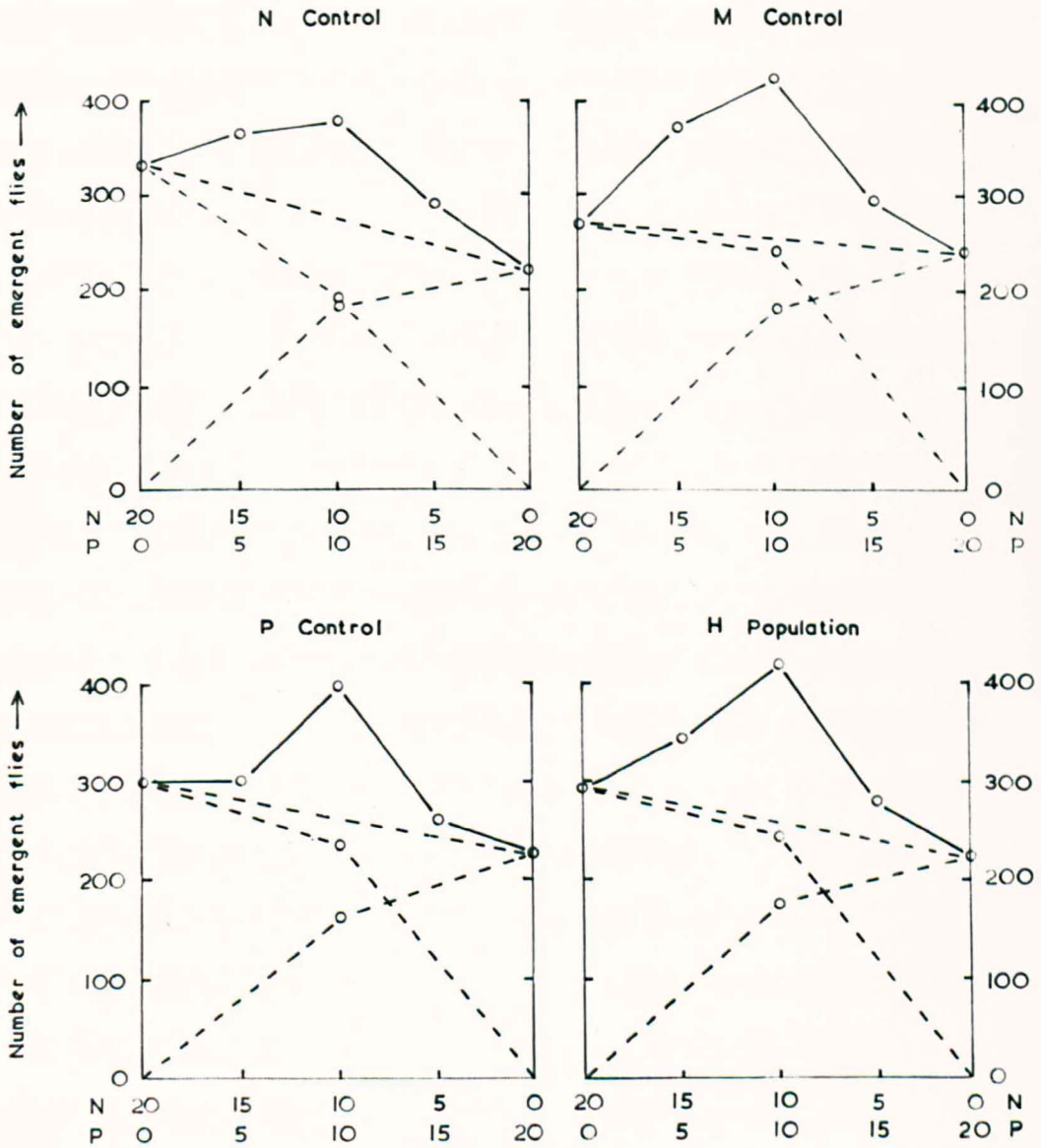


Fig. 37 Results of test 3 - yield on a range of heterogeneity - drawn as Replacement Series Graphs.

(Standard errors in table 12, appendix 2, p 231.)

and peppermint food were available, was shown by all the populations and may be partially exaggerated. The normal- and peppermint-containing tubes only in the $H\frac{1}{2}$ test situation were separated after egg-laying and not those of the controls (see p 124 for explanation). Therefore the larvae in these separated tubes were at a lower density per container than those in the other test situations (20N : 0P, 15N : 5P, 5N : 15P and 0N : 20P). However, by extrapolation of the results on 20N : 0P and 15N : 5P and also on 5N : 15P and 0N : 20P, a peak at $H\frac{1}{2}$ (10N : 10P) was probable in all cases, and may be higher for the heterogeneous population (H).

It should also be noted that the yield on normal food (20N:0P) was greater than on peppermint (0N : 20P),in the ratio of approximately 1.3N : 1P, for the M and P controls and the heterogeneous population (H), (1.5N : 1P for the N control), reflecting the results of tests 1, 2, 4 and 5, reported previously, pp 139 - 145

The interpretation of the De Wit diagrams is, therefore, that, at the start of the experiment, the 3 control populations and the heterogeneous population show more adaptation to normal food than to peppermint and, in addition, show a positive response to the heterogeneous situation.

b) Test 6 - Response to heterogeneity after 17 generations

When the control populations (N, M and P) were tested for response to heterogeneity after 17 generations of selection in a uniform environment, the total number of emergent flies was greater than expectation in the heterogeneous test situations, as in test 3. (Compare total population size with expected population size in fig. 38a). Expressed as biomass, with which there is no comparable figure in test 3, this same result was obtained (fig. 38b) and would indicate that the initial response of a population to a

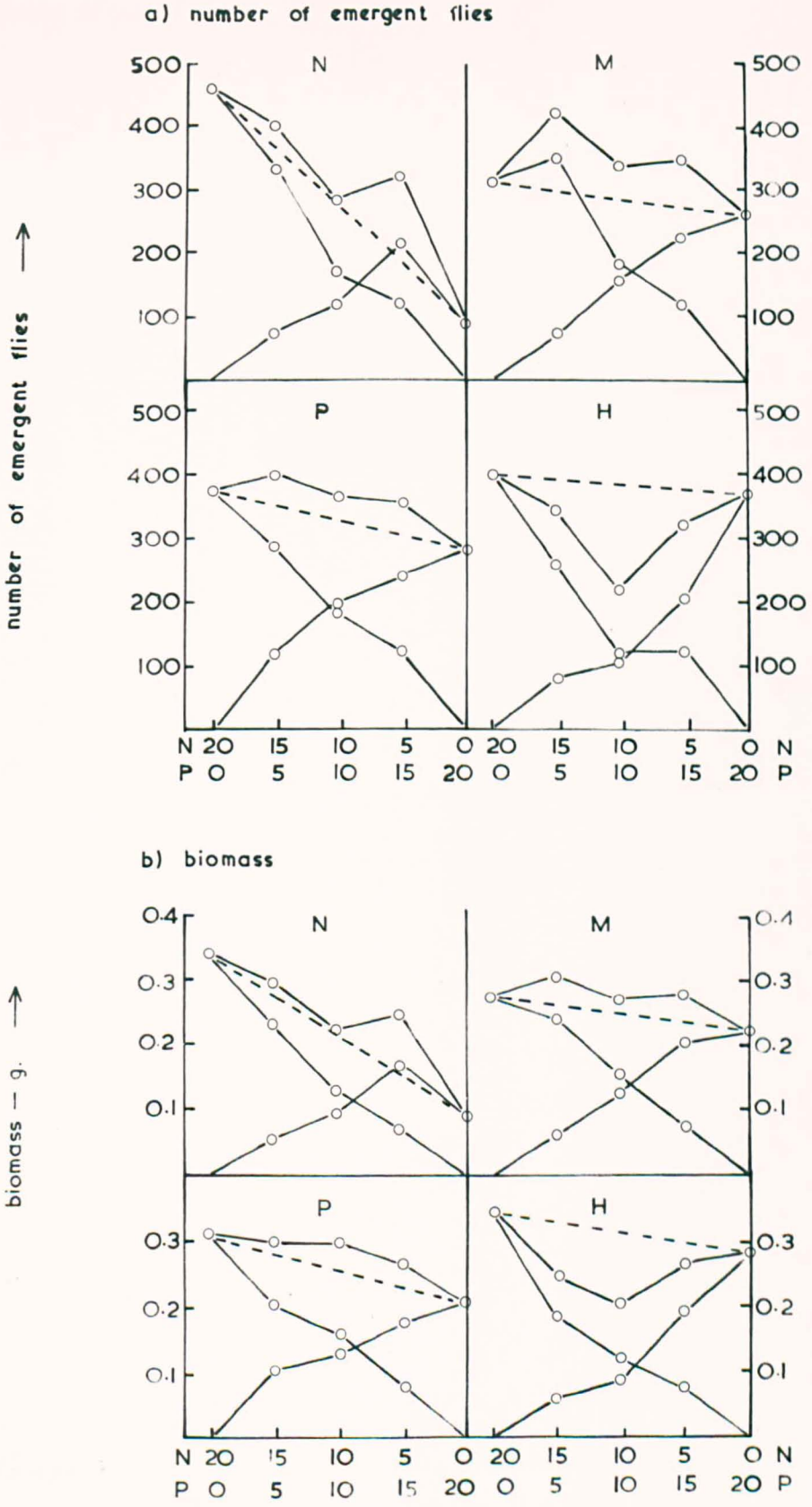


Fig. 38 Results of test 6 - yield on a range of heterogeneity. (Standard errors in table 13, appendix 2, p 232.)

heterogeneous environment is a population size exceeding expectation. (See also results i, fig. 31, p 137, where the biomass of the H population exceeds expectation early in the experiment.)

However, a very different result was obtained for the population reared in the heterogeneous environment for 17 generations, i.e. the H population. All replicates showed a significant deficit of flies, both in numbers and biomass, for the 10N : 10P test situation, compared with the yields in the test controls (20N : 0P, 0N : 20P). Although replicates within treatments (Appendix 2, tables 13a & b) for all tests generally varied too much to allow any quantitative conclusions to be drawn, the decreased yield of the H population in this test was shown markedly by all the replicates (χ^2= 67.7, $P < 0.001$). It should be remembered that in results i, fig. 31, p 137, showing population size over successive generations, biomass of the H population began to decrease from generation 13 and was occasionally below expectation.

The peppermint (P) and half-strength peppermint (M) controls yielded more flies on normal than on peppermint food, in the ratio 1.3N : 1P, which is comparable to that in test 3, indicating no change in adaptation to normal food during the experiment in these controls. The N control population, however, yielded many more progeny on normal food than on peppermint,(4N : 1P), indicating a change in adaptation to normal food within this control. (See also results i, fig. 30, p 136, where the N control showed a steady, significant increase in biomass throughout the experiment.)

The ratio diagrams (fig. 39) show that normal food generally contributed greater numbers and biomass to the resultant population than the peppermint food. The proportion was greater for the N

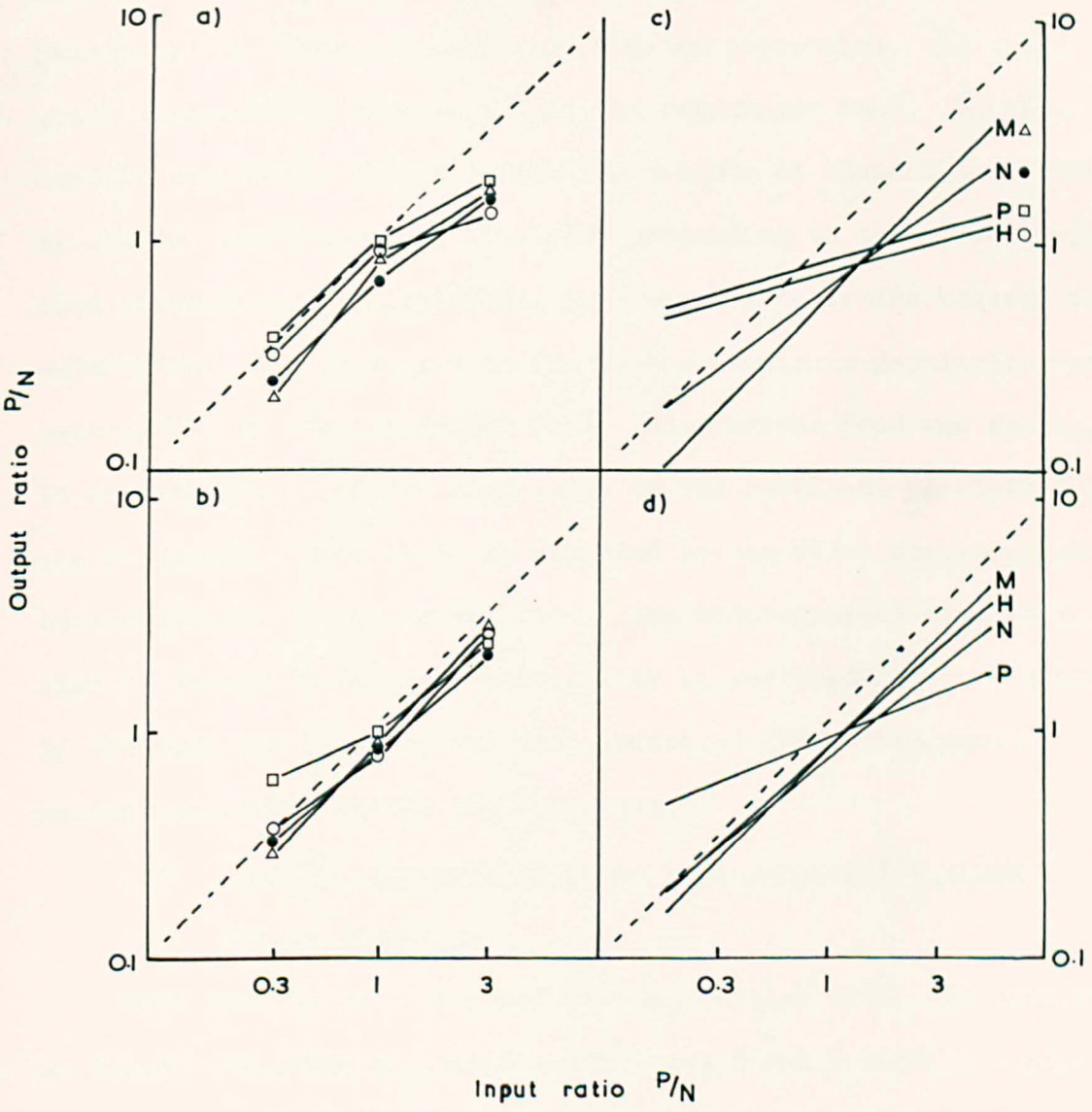


Fig. 39 Results of test 6 drawn as ratio diagrams (log scale).

a) Number of emergent progeny

b) Biomass

c) Regressions of number of emergent progeny

d) Regressions of biomass

control population, whatever the proportion of the two foods, than for the P and M control populations and this indicates a greater adaptation to the normal food within the N control, i.e. evidence of directional selection. In the peppermint (P) control, however, there is evidence that, although not fully adapted to peppermint in terms of increased yield on peppermint, the flies could discriminate between normal and peppermint food. In this control population (P), the relative amounts of biomass contributed by the two foods were not in direct proportion to the amounts of food provided. When peppermint food was scarce in the heterogeneous environment, a greater proportion of the resultant population was contributed by the peppermint food. When normal food was scarce, it contributed a greater proportion to the resultant population in the P control. This is to be expected as the flies always appeared better adapted to the normal food. The heterogeneous population (H) also responded to the heterogeneity by an overproduction of flies in the minority habitat, and this indicated the development of habitat selection within this population.

c) Test 7 - Residual response to heterogeneity after lapsed selection

When exposed to a range of heterogeneities after one generation of lapsed selection on normal medium, the N and M control populations yielded greater-than-expected numbers of progeny and biomass in the heterogeneous situations (see Replacement Series Graphs, figs. 40a & b). This result is comparable to that of test 6 for these populations. The P control population yielded fewer progeny and lower-than-expected biomass in the 10N : 10P heterogeneous test environment. Interpretation of this result is difficult as flies from this population had been subjected to peppermint for 17

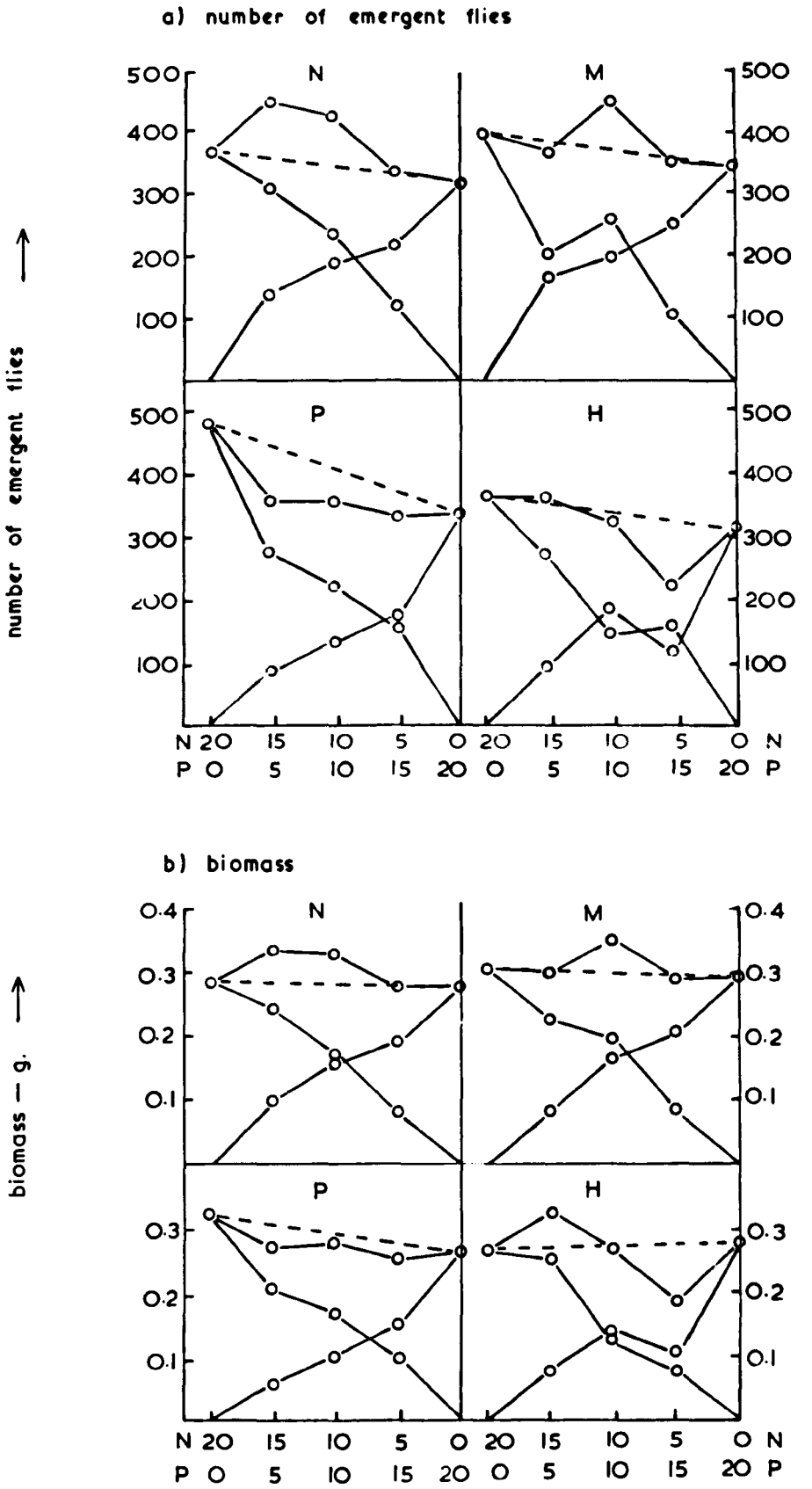


Fig. 40 Results of test 7 - yield on a range of heterogeneity after 17 generations of selection and 1 generation of lapsed selection. (Standard errors in table 14, appendix 2, p 234.)

generations, normal medium for 1 generation and then heterogeneity. The H population yielded more flies (numbers and biomass) in the heterogeneous situation (10N : 10P) than in test 6, but, taken over the three different heterogeneity levels, the yield was still less than expectation. At 5N : 15P heterogeneity level, fewer flies than expectation were produced.

The results are shown more clearly by the ratio diagrams (figs. 41a, b, c & d). The overproduction of flies in the minority habitat, noted in test 6, persisted for flies in the heterogeneous (H) population but was not so marked for flies of the P control population.

Summary

The initial response to heterogeneity, shown by all 3 controls and the H population, was to yield a population size in excess of expectation. After 17 generations of selection, this response was still given by the 3 controls but the yield of the heterogeneous (H) population (both in numbers of emergent progeny and biomass) was significantly less than expectation. This persisted after a generation of lapsed selection, implying that it was a genetic response to the heterogeneity. Analysis of tests 6 and 7, by ratio diagrams, revealed that for flies selected in a heterogeneous environment there was an overproduction of flies on the minority habitat. This indicated discrimination and food choice by the flies and persisted after a generation of lapsed selection, suggesting that it was a possible genetic response to the heterogeneity. There was also evidence of discrimination and food choice by flies selected on peppermint, though it was not a persistent effect.

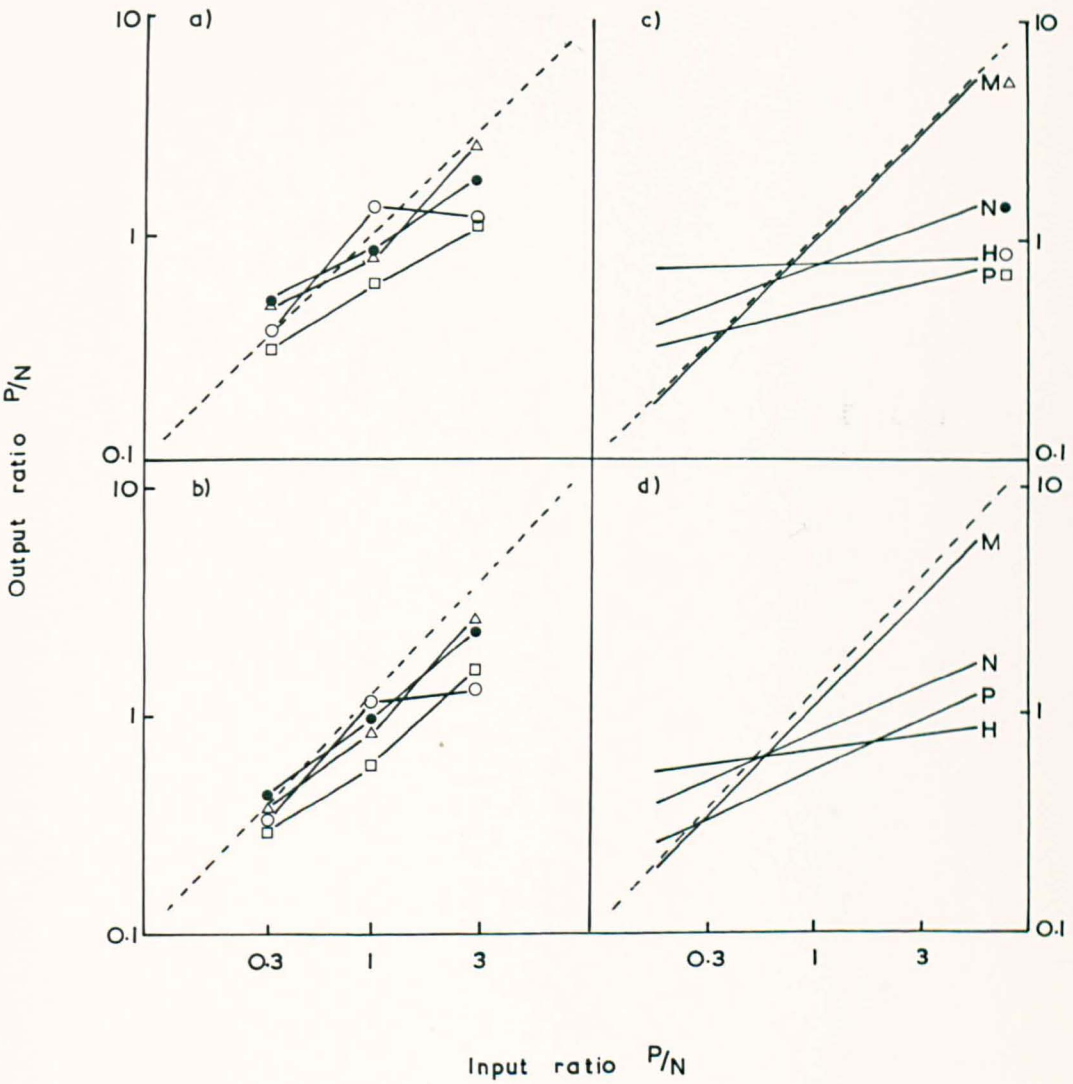


Fig. 41 Results of test 7 drawn as ratio diagrams (log scale).

a) Number of emergent progeny

b) Biomass

c) Regressions of number of emergent progeny

d) Regressions of biomass

iv) Tests for habitat selection

a) Test 8 - Production and distribution of eggs on the different foods after 19 generations of selection

Females from each of the main selection lines of the experiment, N, M, P and H, were tested for egg-laying performance on the food on which they had been reared. The results, detailed in Appendix 2, table 15, p 240, are summarised in the following table:

Table 19. Egg-laying performance

	Treatment				Within het. env$^{t.}$ of H population	
	N	M	P	H	HN	HP
Average no. eggs laid by 20 ♀♀ reared on the indicated food for 19 generations	299.5	306.4	228.0	310.3	265.7	53.0

The numbers of eggs laid by females from the different main selection lines were not significantly different from each other, (Student's t test: t = 2.55 between H and P lines), although fewer eggs were laid by the females of the P control population.

Comparing the number of eggs laid on N and P food, within the heterogeneous environment, by females from the H population, significantly more eggs were laid on normal (266 eggs) than on peppermint food (53 eggs), i.e. 5N : 1P, ($t = 8.5$, $P < 0.001$). Comparison with the number of eggs expected on these foods (calculated from the controls) showed that a significantly greater number were laid on normal food ($\chi^2 = 89.9$) and a number less than expectation laid on peppermint food ($\chi^2 = 32.6$).

b) Test 9 - Habitat selection

At 20 generations, the flies were tested for any habitat preference which might have evolved in the flies subjected to the heterogeneous environment. Extra beakers of N, M, P and H treatments were set up from the main lines of generation 18, as shown in fig. 25, p 122. HP and HN tubes were separated after egg-laying in the H test treatment and in each of the N, M and P controls, 10 tubes were separated at random at that time. In test 9, the females emerging from these separated tubes were tested on N, M, P and H food, separation of the tubes after egg-laying occurring as before. The results are given in table 20, which shows the combined total number of emergent flies from the separated tubes of N, M, P and H test treatments, but also gives the separate totals for flies emerging from the N and P food within the H treatment (HN and HP respectively).

Table 20. Distribution of emergent flies after 20 generations of selection

Test treatment	Medium upon which ♀♀ were reared				
	N	M	P	H	
				HN	HP
N	446.3	388.3	449.0	465.3	439.5
M	366.0	394.7	389.0	460.3	-
P	337.0	352.3	364.7	375.3	355.5
H	403.7	382.0	411.0	387.0	321.5
(HN)	255.7	214.3	233.0	219.7	99.5
(HP)	178.0	167.7	178.0	167.3	222.0
Ratio test treatments N & P.	1.3:1	1.3:1	1.3:1	1.3:1	1.3:1
Ratio on N & P foods in H treatment.	1.4:1	1.3:1	1.3:1	1.3:1	0.4:1

Comparison of the total number of emergent flies from the N and P test treatments, where no choice of egg-laying medium was given, shows that a ratio of 1.3N : 1P was obtained within every line, regardless of the medium on which the mothers were reared. The number of flies emerging from the M test treatment was, in all cases, intermediate between the numbers on the N and P test treatments. Where the females were confronted by a choice of egg-laying medium as in test treatment H, flies from the control lines N, M and P, responded as when no choice was given. The numbers of emergent flies from HN and HP tubes were in the ratio 1.3 : 1 respectively. Flies reared on normal medium within the H line (HN females) also responded in the same way, a ratio of 1.3N : 1P being obtained within the H test treatment. However, those flies reared on peppermint medium within the H population (HP females) responded very differently, more flies emerging from peppermint food than from normal food in the H test treatment ($P < 0.001$). The preliminary experiment 2:2, p 112, showed that eggs once laid survived equally well on either food. Assuming this to be the case after 20 generations of selection, the result of test 9 indicates that a choice of food medium for egg-laying sites was being made by at least the HP females in the heterogeneous environment.

4. General Discussion

i) Adaptation to the experimental system

Ayala (1968) suggested that a genetically variable population, when first subjected to an environment in which resources of food and space are limited, would increase in numbers and/or biomass by exploiting all the niches within the environment and reducing intraspecific competition between the members of the population. Such adaptation was observed in the present experiment. Prior to the experiment, the flies were kept in bottles containing 60ml food. Under experimental conditions, the flies were kept in 250ml beakers containing only 13.2ml of food distributed in 20 small tubes. Therefore there was a limitation of food and space which the flies had not previously encountered. During the experiment, in the three controls and in the experimental population, there was a rapid increase in population numbers and biomass for the first four generations. This would indicate that the flies were exploiting and adapting to their new environment - the type of container, the distribution of food, external factors within the incubator and competition due to differing population densities. It is also evidence of genetic variability within the stock.

Large fluctuations in numbers, from one generation to the next, were noted during this period and throughout the experiment. This was probably due to two different density effects operating within the populations: firstly, the higher the density of adults, the lower the average weight of their offspring because of the limitations of food and space (p 133); secondly, the smaller the female, the fewer offspring she produces. These two factors are probably the cause of the large fluctuations in numbers from one

generation to the next, however constant the environment. Therefore, a large population of small flies would be followed by a smaller population of larger flies (Shorrocks 1970). In addition to these effects, as the density of the adults increases in a limited environment, so there is higher mortality of their offspring at all stages of development. When females are adapted to egg-laying on a medium, they tend to lay large clusters of non-randomly distributed eggs, laying them where other females have laid theirs (Manning, pers. comm.). High mortality of the eggs could be caused 1) by the females trampling on the eggs already laid or 2) by competition between the densely-packed eggs for resources at the larval and pupal stages. Therefore, as adults become better adapted to an environment and lay more eggs, the population numbers may reach an optimum, reflecting the limits of the environment on the population size.

By generation 10, all 4 populations had increased significantly in numbers and an optimum population size of approximately 300 flies had been reached by the normal control (N), the half-strength peppermint control (M) and the heterogeneous population (H). This size of population was never attained by the peppermint control (P) (see p 129). However, its optimum population size of approximately 250 flies was reached at about the same generation and the 4 populations were not significantly different from each other in size. Since this optimum was at a lower level and the size of the peppermint control population was lower than that of the other 3 populations on a significantly greater number of occasions, it is suggested that, for some reason, e.g. desiccation, the peppermint food was incapable of sustaining greater numbers of flies so that the limiting density of the adult population on

peppermint food was lower.

The population size of all 4 populations was not maintained but decreased in successive generations after generation 12. The populations did not fall to their initial levels during the length of the experiment but were no longer significantly different from them. As this effect was shown by all 4 populations, it could be suggested that this decrease in numbers was due to inbreeding depression in the CB7 stock. Ford (1972), investigating ecological divergence between competing populations of the same stock of flies, also found a reduction in population size after 10 generations. He attributed it to a high degree of inbreeding caused by bottleneck effects in the laboratory culture together with inbreeding arising during the experiment, in which only 10 pairs of flies were carried over to form successive generations. In the above experiment however, the laboratory stock might not have been as inbred as Ford's, since the experiment was begun two years earlier. On the other hand, 20 mated females were used to found successive generations and the populations averaged only about 150 females - much smaller than those of Ford. Consequently, there may have been a degree of inbreeding during the experiment although steps were taken to minimise it. Each population was housed in two population cages and successive generations were founded with 10 females taken from each. Latter and Robertson (1962) found that a strong directional selection combined with inbreeding increases the rate of inbreeding depression. Therefore, as there appeared to be little selection pressure operating on the peppermint food and a comparatively stronger selection force operating on the normal food (see pp 139 - 155), it might be

supposed that inbreeding depression would first appear in the normal control population, but this was not found. On the other hand, this could be explained if the extent of inbreeding in the peppermint control population were relatively greater because the population size was generally smaller. However, these considerations take only the numbers of flies into account.

When the biomass of the four populations was compared, no reduction in yield was found. The normal control (N) and the heterogeneous population (H) increased significantly in biomass throughout the experiment and the half-strength peppermint (M) and the peppermint (P) controls remained relatively unchanged - a non-significant increase in biomass being observed. This increase in biomass, in the N and H populations, could be due to the decrease in numbers, resulting from inbreeding depression, giving rise to less dense populations and, therefore, increased resources of food available to individual flies in these populations. This could imply that the effect of inbreeding depression was predominantly at a pre-emergence stage. Information on the numbers of eggs laid by the females would have revealed whether the decline in numbers was due to inbreeding depression (fewer eggs being laid) or whether it was a density effect. However, it was not possible to obtain such information in this experiment. By day 6, when the females were removed, some of the eggs laid on normal food would have already developed into larvae whereas those laid on peppermint would not. In future experimentation, the females could be removed a day earlier, enabling egg counts to be made.

Although inbreeding depression, resulting from increased homozygosity in the population, might give rise to a decrease in

numbers, it does not affect the 'fitness' of the individual when only the food to which it is adapted is available. Whatever the reason for the decrease in numbers, the biomass increased on the normal food and in the heterogeneous environment over several generations and would indicate an improved adaptation to those conditions.

ii) Non-adaptation to peppermint

One of the results of the experiment is the apparent inability of the CB7 stock of D. melanogaster to adapt genetically to peppermint. This failure after 15 generations of selection poses two questions: 1) Was the original stock sufficiently genetically variable? 2) Was the peppermint acting as a selective agent?

It is difficult to assess whether sufficient genetic variability to cope with peppermint resistance existed in the CB7 stock. The stock was chosen because its history suggested it would be relatively outbred and Antonovics (pers. comm.) showed that it was genetically variable for sternopleural chaeta number. Significant family differences in fertility were found in experiment 2:3 (p 115) and, more importantly, there was evidence that the families differed in their response to peppermint. However, genetic variance for response to peppermint appeared to be low, particularly in relation to genetic variance for overall yield. This is borne out by changes in overall productivity in all environments in the main experiment. In addition, the high density conditions of the experiment may have precluded the development of peppermint resistance if this in any way affected the viability of the flies.

Throughout the experiment it was generally observed that the yield on peppermint was lower than on normal food. Flies reared on peppermint for 15 generations did show an increased yield (test 4, p 141) but this disappeared after one generation of lapsed selection (test 5, p 145). Therefore the CB7 stock failed to adapt genetically to peppermint - a toxic substance. However, the peppermint concentration used in the experiment was not toxic to the adult females but caused them to lay fewer eggs (experiment 2:2, p 112). It was not possible to use highly toxic concentrations since it was necessary to ensure the production of a population of more than 50 flies in all the population cages to maintain the same density (20 females) of the founder population in each generation. Therefore, it might be supposed that the selection pressure imposed by peppermint in the experiment would be less than directional selection imposed on the adults by toxicity. The corresponding adaptation to peppermint, shown as an increase in yield, would be slow to build up in the population.

Adaptation to peppermint might have shown itself in ways other than an increase in population size. Peppermint appeared to retard the development of the life-cycle by 1-2 days (pers. comm.) but the effect of peppermint on the physiology of the flies was not investigated. The egg-laying performance of individual flies reared on peppermint was tested (tests 8 and 9, pp 157 - 159) and found to be not significantly different from that of flies reared on conventional food when both were tested on normal food. Flies reared on peppermint, however, were larger in size than those reared on normal medium and the better fecundity of the peppermint females on normal medium in test 6 might be attributable to this. A highly significant correlation did exist between the density of

a population and the weight of the flies in it, regardless of the type of food (p 133), and since the populations on peppermint were generally at a lower density, it is probable that the size effect was not an adaptation to peppermint.

It was suggested that the reduction in the numbers of progeny, caused by peppermint, was due to the females being deterred either from mating or from egg-laying (p 114). Which of these behavioural responses was the more likely was not investigated but would be of interest for future research. As peppermint at low concentration appeared to affect the behaviour of the flies, it is possible that adaptation to peppermint would be shown by a behavioural change in the females, such as the development of a preference for peppermint for egg-laying sites, 'phenotypic flexibility'. Given selection over many generations, it is probable that any such behavioural response would have a genetic component, although it might be slow to evolve. The peppermint control (P) did not experience conditions in which such a behavioural change could be detected until generation 17. When confronted with a choice of food in tests 6 and 7 (pp 149, 153), there was an overproduction of flies from the minority habitat, indicating that the females were discriminating the foods and choosing peppermint when it was in short supply. This persisted after a generation of lapsed selection, implying a genetic component. However, when equal amounts of normal and peppermint food were available to the peppermint control population (test 9, p 158), the normal food was preferred and there was no evidence of a behavioural change in response to peppermint. Therefore, the results were inconclusive.

For future experiments on sympatric divergence, it might be better to employ a different substance to provide a force of

directional selection in the heterogeneous environment. Many instances of selective resistance to different toxic substances have been recorded, e.g. EDTA - (Robertson 1966), NaCl - (Waddington 1959), and the physiological effects of these substances on the flies are well-documented.

iii) Adaptation to the 'normal' food

It was assumed that the CB7 stock of Drosophila was fully adapted to the normal food because the flies had been living on it in laboratory culture. However, there is evidence to suggest that there was an improvement in genetic adaptation to normal food during the experiment. 1) It was found in preliminary experiment 2:3, p 115, that the CB7 stock was highly variable for fertility differences on normal food. 2) At the end of the main experiment, the N control population was significantly greater in biomass than at the start and this was the result of a steady increase from one generation to the next (fig.30, p 136). 3) As an incidental result from the test for response to heterogeneity (test 6, p 149), a much greater yield on normal food than on peppermint food was produced by the N control population (4N:1P) than by the two peppermint control populations, M and P, (1.3N:1P). These latter populations did not differ in their response from that in the earlier test 3 (p 141), when the yield on normal food compared with that on peppermint food was in the ratio of 1.3N:1P. The N control population in test 3 yielded population sizes in the ratio 1.5N:1P. Although the N control population was not tested for yield on normal food after a generation of lapsed selection on another food, which in future experiments would be desirable, it

would not be unreasonable to suppose that the flies were responding genetically to some factor of selection provided by the normal food.

It is possible that this response could have been elicited by a change in the constituents of the food. The normal food at Stirling, based on Mittler and Bennett's (1962) recipe, incorporated nipagin as a fungicide instead of propionic acid, which may have been used when the flies were originally cultivated at Bangor. However, the flies had been living on the food for several generations prior to the experiment and nipagin is not known to have any toxic effects. An alternative explanation is more probable. The populations living on normal food were generally larger in number than those living on peppermint (P control) and therefore more dense. If the flies were well adapted to the normal food, there would be a greater number of eggs laid and, consequently, high mortality of the pre-emergence stages of the life cycle (see discussion, p 161). Under these circumstances, there might well be selection for those individuals better able to withstand the effects of overcrowding. The improved adaptation shown by the population living on normal food might reflect a genetic improvement in co-existence.

iv) Adaptation to the heterogeneous environment

a) Initial response of populations to heterogeneity

The initial response of the 4 populations to heterogeneity was a yield greater than expectation, both in numbers and biomass (see figs. 23 and 24), irrespective of the food on which the flies were reared. This environmental response is perhaps not surprising since it is probably unjustifiable to assume that the media are

independent of each other and that the expected yield is the mean of the yields on normal and peppermint medium alone. For example, it is probable that flies on normal food were subject to high larval mortality whereas flies on peppermint food were deterred from egg-laying. Larval mortality resulting from high larval density on normal food would be reduced by some of these larvae crawling to the peppermint food with a lower larval density and where they can readily survive (preliminary experiment 2:2, p 112). Hence the yield in the heterogeneous environment would be greater than expectation.

b) Population size over successive generations in a heterogeneous environment

The experimental population (H) showed the initial environmental response seen above, i.e. a yield greater than expectation, when first put into a heterogeneous environment. However, while experiencing heterogeneity over successive generations, the yield of this experimental population continued to increase steadily above expectation until generation 10 (fig. 31, p 137). It is possible that this represents genetic adaptation to the heterogeneous environment and it is the predicted result of this experiment. Genetic adaptation of a population, limited in resources, may be revealed by an increase in productivity (Ayala 1968) and Threlfall (unpubl. data) in a similar experiment also noted such an increase in yield. Ford (1972) found that genetic change in competitive ability between the niches in a two-niche situation may give rise to an increase in population size. Equally, however, this increased yield may have been a continuation of the environmental response. It did not persist and by the end of the experiment, the population size and biomass in the heterogeneous environment were less than

expectation. The individual flies, however, did increase significantly in size throughout the experiment, whereas those of both peppermint controls (P and M) did not (p 133). Flies in the N control population also increased significantly in size and this would suggest that the exploitation of and adaptation to the heterogeneous environment could be due to all, or part, of the population improving in response to the normal food.

c) Population stability in heterogeneous environments

Another effect of the heterogeneous environment was to endow the population with greater stability. There are periodic fluctuations in yield from generation to generation in all the populations but these were not so marked in the heterogeneous environment. Since these fluctuations are largely in phase, they probably represent cultural and/or environmental variations. A similar effect was found by Shorrocks (1970). He suggested that, when there were fluctuations in numbers despite a constant environment, the regulation of population size may be due to density effects acting on the females. A generation in which adults are overcrowded and probably smaller in size may be followed by a population of larger flies much reduced in number because of low fecundity of the females or high egg mortality (see discussion p 161). The ability of the experimental population to withstand these effects may result from a differential response of the sub-populations on the two media in the heterogeneous environment having an overall dampening effect: e.g. the egg and larval densities in the two media may be different and any effects interacting with density may be out of phase in the two media.

d) Genetic adaptation to a heterogeneous environment

When the experimental population was tested for response to heterogeneity after 17 generations of selection, the yield (which at generation 10 had been in excess of expectation) was significantly less than expectation and this persisted for a generation of lapsed selection on normal medium, implying that it was a genetic response to the heterogeneity (tests 6 and 7, pp 149 - 155).

The model upon which this experiment on sympatric divergence was based relies on two different foods to provide forces of selection acting disruptively upon a population enclosed within a small area. Under such circumstances, the population may be predicted to respond genetically either (1) by showing signs of sympatric divergence into two strains of flies, each adapted to one of the two media, or (2) by producing unspecialised flies equally well-adapted to both media: if the niche differences are small and the tolerance of the population to both niches is large, then the optimum population will consist of monomorphic, unspecialised types, genetically adapted to such a situation (Levins 1962). In either case, the flies would be subjected not only to directional selection on one or both of the foods, but also to selection pressures imposed by the greater complexity of the heterogeneous environment. In particular, different density effects would operate on the two foods and there may be selection to reduce competition both within and between each sub-population inhabiting each food. One would predict that the population size would increase above expectation, i.e. the level calculated from the yields of each food alone, and that as the population becomes better adapted genetically, this increase would be augmented. This was observed in the experimental population until generation 10. At this time, one would have

supposed that genetic adaptation was taking place in the experimental population, resulting in either divergent types or well-adapted, unspecialised flies. However, several generations later, this population showed a highly significant deficit of flies in the heterogeneous environments of tests 6 and 7 (pp 149 - 155) and the yield of the main selection line itself was below expectation. This result was unexpected because one would predict that the experimental population size should not fall below the expected level calculated from the controls. An explanation of this result may be indicated by the second result of major significance to emerge from the experiment.

When the relative amounts of the two foods in the heterogeneous environment were altered to test the population for response to heterogeneity, (tests 6 and 7, pp 149 - 155), there was an overproduction of flies from the minority habitat, though the number of replicates was low. (See ratio diagrams - figs. 39 & 41). This indicates that the flies could discriminate normal and peppermint food and were choosing egg-laying sites. (The tubes containing the two types of food were separated after egg-laying to establish the size of the emergent sub-populations). This result persisted after a generation of lapsed selection, indicating a genetic component, and may have contributed to the first result noted above. It indicates divergence within the population.

Habitat selection by the flies in the heterogeneous population was also independently demonstrated (test 9, p 158) - all females maintaining their preference for normal food except females raised on peppermint food in the heterogeneous environment; these preferred peppermint food on which to lay their eggs. The greater

stability of the heterogeneous population noticed (1) in different pure peppermint concentrations and (2) as less extreme fluctuations in total yields towards the end of the experiment provides additional evidence of habitat selection. This behavioural change, i.e. a preference for the less attractive peppermint food, was exhibited by the heterogeneous population after one generation of lapsed selection on normal food (test 7) and therefore was a genetic change. This genetic adaptation to peppermint, shown not as an increase in yield but as a behavioural change, could be regarded as a form of 'phenotypic' or 'behavioural flexibility' within the population. There was also evidence from tests 6 and 7 that the females from the P control population were showing a preference for peppermint,when placed in a heterogeneous environment in which peppermint formed only $\frac{1}{4}$ of the total food available. However, in the subsequent test of habitat selection (test 9), when equal amounts of normal and peppermint food were available, the preference for peppermint was not maintained. Therefore, the evidence concerning adaptation to peppermint in the peppermint control population is conflicting.

Is it possible to account for the discrepancy between the predicted results and those observed in the experimental situation in terms of sympatric divergence within the population? Consider the experimental heterogeneous environment. The relative proportions of the two habitats, normal and peppermint food, remained constant (1N:1P) and the initial response of the population was for the relative yields on the two foods to be in the ratio 1.3N : 1P. Therefore, at the beginning of the experiment, more flies would live on normal food and, therefore, more of these

would be chosen to found successive generations. If, in addition, the flies were to adapt genetically to some factor provided by the normal food, either to the food itself or to the overcrowding on it, then food preferences and habitat selection in the heterogeneous habitat might evolve, even in the absence of adaptation to peppermint. It was found in test 8 (p 157) that $\frac{5}{6}$ of the eggs laid in the heterogeneous environment were on normal food. Since normal food comprised only half of the food available, competition would be severe and density effects would limit the productivity of this medium. Conversely, the $\frac{1}{6}$ of the population on peppermint, would have half the food resources available but, if no adaptation to peppermint had taken place, the productivity would not improve. Therefore, the net result of improved adaptation on normal medium alone would be a yield considerably less than expectation. This was observed both in the heterogeneity test (test 6) and over successive generations towards the end of the experiment. However, this result also persisted after a generation of lapsed selection on normal food, implying that selection was operating on the peppermint food.

It may be erroneous to assume that because there was no adaptation to peppermint in either of the peppermint controls (P or M) there would be none in the heterogeneous environment. In the absence of a suitable genetic test, which should be included in future research, habitat selection, by the sub-populations on normal and peppermint food within the heterogeneous population in test 9, could be explained by phenotypic flexibility. There could also be (1) genetic differences in ability to be phenotypically flexible, i.e. phenotypically plastic, (2) genetic differences in

resistance to peppermint, or (3) both, within the population. The second of these alternatives is unlikely because no resistance built up in the P and M control populations but the last alternative is possible since evolution of stronger habitat selection may be accompanied by some change in resistance. The existence of genetic differences in ability to be phenotypically flexible is a possible explanation.

Because it was assumed that there was no genetic adaptation to peppermint, a test for genetic divergence in the heterogeneous population was not made - separated sub-populations of flies from normal and peppermint food (i.e. HN and HP flies) were not tested on a range of peppermint. This would be desirable in future research. The results of the heterogeneous population tested on a range of peppermint concentration may be the mean of quite resistant and non-resistant flies and the number of replicates may not permit this to be distinguished from all or mostly non-resistant flies.

In the heterogeneous environment, phenotypic plasticity might evolve because it may be an advantage to lay eggs on peppermint, especially if the normal food in the heterogeneous environment is density limited. There would be less wastage of eggs and better exploitation of the whole environment. This might accelerate the eventual reduction in population size if density limits are met.

It is further possible that habitat selection could lead to assortative mating within the population. Peppermint food had the effect of retarding development by approximately one day. This would help to reduce the randomness of the population. Smith (1966) considers that habitat selection is an aid to sympatric divergence.

The genetic build-up of habitat selection for egg-laying and a small amount of assortative mating would offer a measure of increasing isolation, which would tend to reduce gene flow and effectively augment the selection pressure imposed on the population by the heterogeneous environment. In addition to investigating the genetics of habitat-choice, behavioural tests on mating preferences within the heterogeneous population would be of interest for future research.

Even in the absence of directional selection on peppermint, one would suggest that unique selective forces were operating in the heterogeneous environment. These forces appeared to be producing increased habitat selection and bringing about some measure of divergence within the population.

5. Summary and Conclusions

1) Preliminary investigations on Drosophila melanogaster (stock CB7) were carried out to determine the nature of the response to peppermint-adulterated food medium, which is toxic at high concentrations.

2) An experiment was described to investigate the possibility of sympatric divergence arising in a small, randomly-mating population of D. melanogaster due to the disruptive effects of an environment heterogeneous for two different food media, one of which was toxic. Normal food (sucrose, yeast and agar) and peppermint food (sucrose, yeast, agar and peppermint) were used.

3) After 15 generations of selection, control populations reared i) on full-strength peppermint and ii) on half-strength peppermint showed no adaptation to the peppermint food in terms of increased yields. The former control produced fewer progeny than the other controls throughout the experiment.
A third control population showed, by an increase in population size, an improvement in adaptation to normal food. This was possibly a genetic response to the highly competitive conditions pertaining on this densely-populated food medium.

4) As an environmental response on first experiencing environmental heterogeneity, the three controls and the experimental population yielded greater-than-expected numbers of progeny.

5) For the first 10 generations of selection in a heterogeneous environment, the difference between the observed and expected size of the experimental population increased steadily. After

17 generations of selection, however, the population size was below expectation and this response was heritable. When the relative amounts of the two foods were changed within the heterogeneous environment at this time, to test the response of the flies, there was an overproduction of flies from the minority habitat - a heritable response. This indicated the development of habitat choice by the flies, which was independently demonstrated in a subsequent test on the choice of egg-laying sites. Females reared on peppermint chose peppermint on which to lay their eggs, females reared on normal food retaining their preference for normal food.

There was less fluctuation in population size from generation to generation in the heterogeneous environment and it was concluded that the greater complexity of this environment endowed its population with greater stability over time.

6) Complete sympatric divergence into two strains of flies, each adapted to one of the two foods, was not observed, though the population became non-random and some flies may have been better adapted to the normal food. A measure of isolation (habitat choice) did evolve during the experiment. Consequently it is suggested that there are conditions under which sympatric divergence within a small, randomly-mating population would occur as a result of disruptive selection within a heterogeneous environment.

SECTION IV

GENERAL CONCLUSIONS

General Conclusions

Even though heterogeneous environments are the rule rather than the exception in natural conditions, it is only recently that their impact on the genetics of populations and on evolutionary processes has been studied with any rigour. The investigations described here have mainly been concerned with assessing what impact environmental heterogeneity on a very small scale (well within the dispersal range of the organism studied) has on genetic differentiation.

The first study looked at the effect of a heterogeneous pasture (mainly height variations) on ribwort plantains, Plantago lanceolata, in more or less natural conditions. The second study looked at the effect of a patchy environment (normal food and peppermint food) on fruit flies, Drosophila melanogaster, in an experimental situation in the laboratory. In both cases, a significant effect of environmental heterogeneity was detected. In the Plantago population, there was evidence of habitat-correlated genetic differentiation at least in some morphological characters. There was also evidence for a considerable degree of phenotypic plasticity, although it was not clear to what extent this was due to the general characteristics of this highly variable, cosmopolitan species. In the Drosophila experiment, populations in the heterogeneous environment evolved habitat selection for the less palatable habitat (peppermint food), presumably so as to exploit this resource more fully. No genetic adaptation to peppermint was detected (as there was no increase in yield) but the evolution of habitat selection necessitated a behavioural change in the

choice of egg-laying sites by the females (phenotypic flexibility) and this might have a genetic component. It was found that the flies became better adapted to living on normal food and it is postulated that this might be the result of a genetic change in ability to live in the more highly-competitive conditions on that densely-populated food medium. The evolution of habitat-selection in the heterogeneous environment could then be partially explained in terms of this effect. It is therefore suggested, though not proved, that genetic differentiation was evolving in the Drosophila population in the heterogeneous environment. It is likely that the results of any investigation on sympatric divergence would be dependent on the species and population studied. It is interesting that in the Plantago population, where selection pressures were probably high, the adaptation was by microdifferentiation as well as plasticity. The role of environmental heterogeneity in maintaining genetic variation was very evident in this population. Prostrate plantains produced fewer inflorescences, were fewer in number and produced fewer seed per plant. Nevertheless, since there were segments of the environment in which these types were favoured, and in which population size was controlled independently of other segments, prostrate genotypes were maintained. In the Drosophila experiment, where selection pressures on the peppermint food in the heterogeneous environment were relatively low, adaptation to the food appeared to be by an increase in phenotypic flexibility (a behavioural change resulting in the preference of the more unpleasant peppermint food for egg-laying). A genetic response was elicited from the experimental population by the heterogeneous environment. It is suggested that a heritable

component of phenotypic flexibility in response to peppermint, together with a genetic response to overcrowding on the normal food, contributed to the result. Therefore, although not much in evidence, it is suggested that genetic differentiation was evolving in this population due to the heterogeneity of the environment.

In both experiments, forces were seen that might enhance positive assortative mating and thus further contribute to maintaining genetic variation. In the Plantago population, this occurred by differences in flowering time between the genotypes and probably some assortative mating by virtue of clumping of like genotypes; in the Drosophila population by habitat selection combined with differences in developmental time on the two media.

It seems clear that in plants (and many sessile animals) vast quantities of seeds are produced and the resultant seedlings have little choice as regards their habitat. These factors contrive to produce strong selection pressures so that only appropriately adapted types can survive. Given this rather basic interpretation, it seems likely that the kind of differentiation observed in the field study is probably commonplace in plant populations. Recent theoretical models do indeed confirm and generalise these ideas, yet have lacked conviction for want of field observations: such observations are provided by the present investigation.

SECTION V.

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APPENDIX 1.

Plantago lanceolata data

APPENDIX 1

Table 1. Germination of P. lanceolata

No. days after seed sown	Population 1 Germination /56 seeds			Population 2 Germination /56 seeds			Population 3 Germination /56 seeds			Population 4 Germination /56 seeds			Population 5 Germination /56 seeds		
	No.	C.I.*	%	No.	C.I.*	%	No.	C.I.*	%	No.	C.I.*	%	No.	C.I.*	%
4	17	6.9	30.4	36	7.2	64.3	9	5.5	16.0	14	6.5	25.0	17	6.9	30.4
5	22	7.3	39.3	39	6.9	69.6	15	6.6	26.4	20	7.2	35.7	22	7.3	39.3
6	27	7.5	48.2	40	6.8	71.4	27	7.5	48.2	23	7.4	41.1	28	7.5	50.0
7	30	7.5	53.6	42	6.5	75.0	27	7.5	48.2	26	7.5	46.4	29	7.5	51.8
8	33	7.4	58.9	45	5.9	80.4	27	7.5	48.2	27	7.5	48.2	30	7.5	53.6
9	36	7.1	64.3	46	5.7	82.1	31	7.4	55.2	30	7.5	53.6	33	7.4	58.9
10	38	7.0	67.9	46	5.7	82.1	33	7.4	58.9	32	7.4	57.2	37	7.1	66.1
11	42	6.5	75.0	49	4.9	87.5	38	7.0	67.8	37	7.1	66.1	40	6.8	71.4
12	46	5.7	82.1	52	3.9	92.9	43	6.3	76.8	42	6.5	75.0	43	6.3	76.8
13	47	5.5	84.0	53	3.4	94.6	43	6.3	76.8	45	5.9	80.4	45	5.9	80.4
14	47	5.5	84.0	53	3.4	94.6	44	6.1	78.5	46	5.7	82.1	45	5.9	80.4
15	47	5.5	84.0	53	3.4	94.6	44	6.1	78.5	46	5.7	82.1	46	5.7	82.1
16	47	5.5	84.0	54	2.8	96.5	46	5.7	82.1	47	5.5	84.0	46	5.7	82.1
17	47	5.5	84.0	54	2.8	96.5	46	5.7	82.1	47	5.5	84.0	46	5.7	82.1
18	47	5.5	84.0	54	2.8	96.5	47	5.5	84.0	47	5.5	84.0	46	5.7	82.1
19	47	5.5	84.0	54	2.8	96.5	47	5.5	84.0	47	5.5	84.0	46	5.7	82.1
20	47	5.5	84.0	54	2.8	96.5	47	5.5	84.0	47	5.5	84.0	46	5.7	82.1
21	47	5.5	84.0	54	2.8	96.5	47	5.5	84.0	47	5.5	84.0	46	5.7	82.1
22	47	5.5	84.0	54	2.8	96.5	47	5.5	84.0	47	5.5	84.0	46	5.7	82.1

C.I.* - Confidence interval

C.I. = $2\sqrt{pqn}$ (Kolmagoroff-Schmirnoff test).

APPENDIX 1 Table 2 Morphology of populations 1 - 5 after one month's growth in greenhouse. Measurements taken 13.8.68

Rep No	Population 1				Population 2				Population 3				Population 4				Population 5			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	8	6.6	0	2	9	11.4	1	3	7	10.4	0	3	8	11.4	0	3	8	15.0	1	3
2	5	7.1	0	3	9	9.6	0	2	6	9.6	0	3	7	14.7	0	3	6	11.2	0	3
3	9	8.1	0	1	9	12.4	1	1	6	7.6	0	3	6	8.9	0	3	7	11.4	1	3
4	9	5.6	0	1	10	11.7	0	1	9	7.6	0	2	7	10.2	0	3	6	9.6	1	3
5	11	8.1	0	1	7	8.9	0	3	9	8.1	0	2	5	7.6	0	3	7	11.4	0	3
6	9	6.6	0	1	7	11.2	0	3	5	8.1	0	3	6	7.6	0	3	6	6.3	0	3
7	9	4.0	0	1	9	10.9	0	3	3	4.8	0	3	6	7.6	0	3	8	13.7	3	3
8	9	11.2	0	1	9	10.9	2	1	7	11.2	0	3	9	14.5	3	2	6	11.2	0	3
9	11	7.1	0	1	7	10.4	0	2	5	5.3	0	3	9	12.2	0	1	9	10.7	3	1
10	9	6.3	0	1	9	11.2	0	1	6	6.3	0	3	4	7.9	0	3	7	10.4	2	3
11	10	6.6	0	1	7	10.6	0	2	5	9.1	0	3	6	12.5	0	3	5	9.4	0	2
12	9	9.4	1	1	8	14.0	0	3	8	7.6	0	3	7	8.9	0	3	7	9.4	0	3
13	7	5.3	0	1	9	11.9	0	1	9	8.9	0	3	5	5.8	0	3	6	7.5	0	3
14	7	6.6	0	1	8	8.4	0	2	7	10.4	0	3	8	11.7	0	3	7	10.7	2	3
15	9	7.1	0	1	9	8.9	0	1	10	10.1	0	2	10	12.7	0	1	8	9.6	3	1
16	10	6.6	0	1	6	5.8	0	3	8	11.2	0	3	7	7.3	0	1	8	12.2	2	2
17	7	6.8	0	1	8	11.2	0	1	5	8.1	0	3	8	9.6	0	3	6	9.9	0	3
18	6	6.3	0	2	7	9.9	1	1	6	10.9	0	3	8	12.2	0	2	7	8.9	0	3
19	10	6.8	0	1	9	11.7	1	1	7	8.9	0	3	9	13.5	0	2	6	10.4	0	3
20	7	5.6	0	1	11	12.2	0	1	5	7.4	0	3	6	8.9	0	3	5	9.9	0	3
21	8	11.2	0	1	9	10.7	0	1	6	9.6	0	3	7	12.5	0	3	6	6.8	0	3
22	8	8.4	0	3	7	14.5	0	3	7	7.9	0	2	9	11.9	0	1	6	8.4	0	3
23	7	6.8	0	2	7	11.4	0	1	4	5.4	0	3	5	6.3	0	3	6	8.1	0	3
24	10	8.1	2	1	9	6.3	0	1	7	10.9	0	2	6	8.1	0	3	9	13.2	2	3
25	9	8.4	0	1	9	7.4	0	1	4	6.3	0	3	8	10.2	0	3	6	11.4	0	3
26	11	6.6	0	1	9	9.6	0	1	5	5.3	0	3	4	7.6	0	3	8	17.3	2	3
27	9	7.1	0	1	8	11.9	0	3	5	7.9	0	3	8	13.0	0	3	6	18.0	0	3
28	10	7.1	3	1	9	14.7	0	3	5	8.1	0	3	7	13.2	2	3	6	8.9	0	3
29	6	6.3	0	3	9	15.0	0	1	4	8.4	0	3	5	6.6	0	3	6	9.4	1	3
30	7	2.8	0	1	7	10.7	0	1	10	14.0	0	3	5	8.1	0	3	6	11.2	1	3
31	7	6.8	0	2	11	10.7	0	1	9	10.7	0	3	4	8.1	0	3	9	14.7	2	3
32	9	8.9	0	1	10	12.7	1	1	5	13.2	0	3	8	11.4	0	2	7	10.2	2	2
33	9	9.4	0	1	8	15.8	0	3	5	8.1	0	3	4	6.6	0	3	7	12.5	2	3
34	6	5.8	0	3	10	14.0	0	1	8	9.6	0	2	5	6.3	0	3	8	11.2	0	3
35	10	8.6	0	1	8	10.9	1	1	8	14.0	0	3	9	9.1	1	3	8	13.2	1	2
36	11	7.8	0	1	7	11.4	0	3	6	12.7	0	3	4	5.1	0	3	8	14.5	3	3
37	9	5.1	0	1	10	14.2	6	1	7	11.4	0	3	8	13.2	0	3	7	11.2	1	3
38	9	9.6	0	1	6	7.3	0	2	6	13.2	0	3	6	12.2	0	3	7	15.5	3	1
39	6	4.6	0	3	5	6.6	0	2	7	13.7	0	3	4	8.1	0	3	9	12.2	3	1
40	10	6.8	0	1	5	9.5	0	3	9	13.7	0	3	7	14.7	0	3	9	11.9	2	2
41	10	7.1	0	1	9	6.6	0	3	9	11.7	0	3	7	8.1	0	3	5	9.4	0	3
42	6	4.1	0	3	9	11.4	0	2	5	10.9	0	3	5	8.4	0	3	7	9.1	0	3
43	10	7.6	2	1	9	14.2	0	1	7	11.2	0	3	8	10.7	0	3	5	9.6	0	3
44	7	8.4	0	2	10	15.5	0	1	4	5.8	0	3	5	8.9	0	2	6	7.6	0	3
45	9	9.9	0	1	10	10.4	0	1	9	8.9	0	1	8	10.7	0	3	7	7.9	0	3
46	9	4.8	0	1	3	4.8	0	3	8	14.5	0	3								
47	11	7.6	3	1	10	10.9	0	1	5	7.4	0	3								
48					9	12.7	0	3												
49					6	7.3	0	3												
50					8	12.2	0	3												
51					6	8.1	0	3												

Key for headings: A leaf number

B leaf length (cm)

C inflorescence number

D growth habit (1 - 3 scale, 1 being prostrate)

APPENDIX 1 - Table 3 Morphology of populations 1 - 5 after 6 months' growth. Measurements made on 8.1.69

	Population 1				Population 2				Population 3				Population 4				Population 5			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	8	39.2	2.4	2	10	30.1	2.4	2	8	44.5	1.7	3	12	46.2	2.2	3	8	23.0	2.7	2
2	5	45.0	1.8	1	11	39.7	1.9	3	4	47.0	1.8	3	7	51.6	2.1	3	9	43.5	2.1	3
3	9	38.3	3.0	1	9	44.5	2.8	2	5	47.6	2.5	3	6	41.0	1.8	3	10	36.8	1.9	3
4	6	38.5	1.8	2	10	50.5	2.4	3	14	38.1	2.1	2	9	42.5	1.9	3	9	52.0	2.2	3
5	9	46.1	2.4	1	14	46.8	2.7	3	12	43.2	2.9	3	4	34.7	1.9	3	5	30.6	1.5	3
6	8	47.3	3.7	1	7	43.2	1.5	3	4	36.0	1.3	3	7	38.0	1.4	3	6	35.4	1.8	3
7	6	41.0	2.3	3	8	42.6	1.8	2	3	32.5	1.0	3	8	40.5	1.5	3	4	37.0	2.0	2
8	11	43.0	3.0	1	10	44.1	2.6	2	7	50.0	2.5	3	11	42.5	1.5	3	6	30.5	2.5	2
9	11	35.7	2.3	2	14	39.1	2.7	2	6	35.0	1.4	3	14	40.5	3.1	2	6	33.2	1.7	2
10	7	39.2	1.9	2	10	42.6	2.2	3	5	31.5	0.6	3	8	42.9	2.2	3	7	38.2	2.4	3
11	9	34.5	2.3	2	6	42.0	1.9	2	6	45.0	1.8	2	8	46.5	1.8	3	7	32.0	2.2	2
12	8	37.1	2.2	2	10	51.3	2.6	2	5	35.0	1.8	3	7	40.5	1.9	3	13	42.5	1.9	3
13	7	38.5	1.8	1	15	42.1	2.5	1	9	45.0	1.9	3	5	34.4	1.6	3	7	34.5	1.3	3
14	7	41.5	2.8	2	5	33.3	1.3	3	7	47.2	1.8	1	11	44.1	2.6	2	4	28.0	1.3	2
15	7	43.1	1.7	2	7	33.6	1.8	3	14	44.6	2.7	3	12	43.8	1.8	2	5	41.1	1.4	3
16	11	41.0	2.2	2	6	37.5	1.4	3	10	47.5	2.3	2	11	27.5	1.9	1	6	30.5	1.5	3
17	7	41.5	4.2	3	8	34.3	2.0	3	5	33.0	1.0	3	8	37.6	1.6	3	7	29.5	2.6	2
18	6	39.0	1.8	2	6	37.2	2.2	3	8	48.0	2.0	3	9	44.2	2.3	2	4	36.5	1.6	3
19	11	44.5	3.2	1	12	46.6	2.3	3	12	44.2	2.5	3	13	42.8	2.0	2	6	33.7	2.3	2
20	7	25.5	2.2	1	15	51.0	2.5	3	6	28.9	1.0	3	7	42.2	2.5	3	4	35.4	2.3	2
21	12	38.5	4.3	1	19	39.6	3.8	2	7	41.4	2.0	1	8	36.5	1.6	3	8	28.3	1.5	3
22	8	38.0	1.7	2	4	25.6	1.3	3	9	35.9	2.2	2	10	41.0	2.8	1	10	27.0	1.7	3
23	6	36.0	2.0	3	12	39.1	3.0	3	4	34.0	0.9	3	6	22.3	1.5	3				
24					11	46.0	2.8	2	9	46.0	2.4	2								
25					9	32.8	2.1	2	7	36.0	1.8	3								
26					14	38.5	1.5	2												
27					9	35.0	2.5	3												

Leaf length and leaf width in centimetres
Growth habit on a 1 - 3 scale

Key for heading: A = Leaf No.; B = Leaf length
C = Leaf width; D = Growth habit

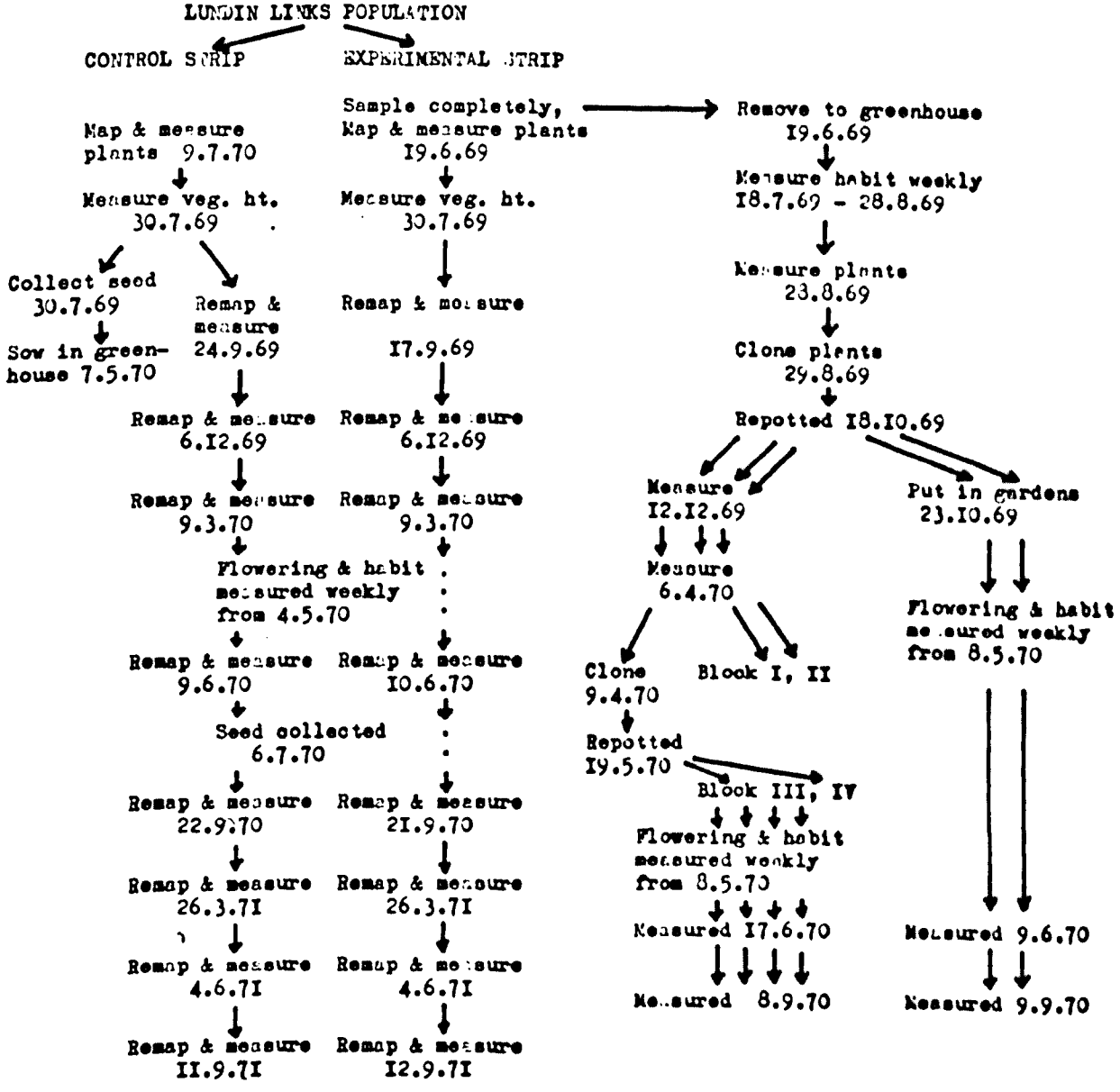
Table 4. Response to shading.

	Prostrate unshaded population				Prostrate shaded population				Erect unshaded population				Erect shaded population			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Box 1	6	22	235	4	6	13	179	2	4	8	250	3	8	18	210	2
	7	14	204	1	6	17	238	3	3	8	230	3	10	15	176	3
	5	10	170	1	4	12	153	3	7	18	343	2	4	9	92	4
	8	16	196	2	7	15	231	2	5	9	318	3	6	14	207	4
	10	23	178	2	3	9	181	3	3	7	241	4	5	9	168	4
	5	17	206	2	3	9	136	2	5	13	327	3	5	12	183	4
	12	15	105	1	7	17	228	2	9	16	385	2	8	16	204	3
	4	12	171	3	6	23	271	3	2	18	350	3	11	25	356	2
	5	7	110	2	4	12	255	3	2	10	346	3	11	15	232	3
	5	13	170	2	5	13	200	3	6	12	304	4	6	15	225	3
	12	24	246	2	8	13	141	2	5	7	202	2	4	8	135	4
	4	8	160	3	8	14	190	3	8	22	336	2	9	16	290	3
	8	20	199	2	9	14	190	3	3	9	281	4	6	13	232	3
	8	24	168	2	4	10	183	4	13	16	340	2				
	4	10	136	2	10	24	188	2	7	15	275	2				
	8	11	125	2	10	18	200	2	7	19	362	2				
	6	20	197	2	5	10	182	3	4	10	333	3				
	21	25	158	2	6	18	204	2	6	13	336	2				
	6	13	166	2	7	15	207	3	4	13	220	3				
	16	18	143	1	9	16	241	2	9	20	395	2				
	8	20	225	1	19	22	246	2								
	5	13	172	2	7	16	262	2								
	4	10	169	2	7	14	232	2								
					2	12	116	3								
					6	19	221	2								
Box 2	11	15	175	2	6	12	305	3	6	14	276	3	9	14	309	3
	8	14	160	3	6	17	360	2	4	8	222	3	7	19	312	2
	5	11	99	2	6	18	300	3	7	11	241	4	4	14	242	4
	6	14	116	2	6	12	369	2	5	9	216	2	5	12	300	3
	5	9	100	2	9	13	297	3	4	11	225	3	4	14	285	3
	7	17	147	2	11	17	280	2	3	6	122	3	14	18	273	2
	6	12	121	2	4	11	297	4	4	10	217	3	5	9	236	4
	6	7	101	1	5	13	232	4	5	10	188	2	4	12	310	3
	10	21	151	1	8	13	242	2	7	12	340	2	6	6	316	4
	5	14	94	2	4	15	227	4	10	15	226	2	3	7	243	4
	10	15	146	2	16	19	273	2	5	9	265	4	10	24	336	2
	4	13	167	4	7	15	267	3	6	13	286	2	2	5	210	4
	7	13	76	1	2	7	158	3	10	19	330	2	5	17	327	3
	4	6	52	1	7	20	237	2	3	7	163	3	4	17	317	2
	4	7	106	3	4	9	206	3	3	6	150	4	2	7	201	4
	5	12	102	2	9	20	275	2	10	11	267	3	7	14	303	2
	5	13	86	2	3	7	180	3	5	12	210	3				
	13	16	193	2	13	17	225	3	5	10	162	4				
	6	10	72	2	8	17	232	2	6	11	285	3				
	8	14	84	1	8	25	230	2	5	11	187	3				
	10	18	150	2					7	6	123	3				
	9	18	93	2					6	10	143	4				
	4	10	91	2					7	12	222	3				
	12	16	79	1					8	14	198	2				

Key to heading: A = Leaf number, B = Leaf width (mm), C = Leaf length (mm), D = Growth habit (1 - 5 scale).

APPENDIX 1

Fig. 1. Experimental schedule of the Plantago lanceolata investigation.



APPENDIX 1

Cloning technique

It was reported (McNeilly, pers. comm.) that plantains could be cloned by removing the leaves from the vertical rhizome by a horizontal incision more or less at ground level. This resulted in the production of numerous side shoots, which could be removed and propagated. To investigate this further and to devise a method for cloning the experimental plants from Lundin Links, an experiment was designed to test the efficacy of alternative methods of cloning.

Plants taken at random from the population comparison experiment, and which had been growing in the greenhouse for one year, were divided into 5 groups of 10 plants. For each group a different type of incision was made through the apical meristem. These incisions were as follows:

1. Vertical incision through rootstock,
2. transverse incision above top of rootstock,
3. transverse incision midway down rootstock,
4. transverse incision near bottom of rootstock,
5. scoop incision.

The plants were potted up in John Innes No 1 potting compost in 5in pots and arranged in random order in the greenhouse. After one month, the number of shoots per plant and the number of plant deaths were recorded (table 5).

Since there was a similar amount of variation in the clone size produced per plant for all the methods tried, it was decided to use method 3 by which to clone the plants. This method produced the highest average clone size. Method 1 did

induce a reasonably high number of shoots per plant and no deaths resulted but the shoots were of unequal size. It was preferred to sacrifice a few plants in favour of uniformity of replicates.

Table 5. Results of cloning experiment

	No. shoots produced using incision method number:				
Rep. no.	1	2	3	4	5
1	4	3	0	15	10
2	10	1	5	12	1
3	3	8	10	7	3
4	1	3	10	1	5
5	12	2	6	7	2
6	6	1	10	4	2
7	4	9	11	5	1
8	13	3	20	15	1
9	7	7	0	0	6
10	2	2	10	15	3
Ave. no. shoots	6.2	3.9	8.2	8.1	3.4
No. deaths	0	0	2	1	0
Coeff. variation	0.68	0.76	0.72	0.72	0.85

Table 6. Pattern analysis - transformed data

Block size	Sx^2	Sx^2/Block size	Sum Sq.	dF	Mean Sum Sq.
1	91.581	91.581	32.987	1279	0.026
2	117.188	58.594	13.598	639	0.021
4	179.982	44.996	5.907	319	0.019
8	312.709	39.089	7.244	159	0.046
16	509.515	31.845	3.458	79	0.044
32	908.394	28.387	1.777	39	0.046
64	1703.052	26.610	1.517	19	0.080
128	3211.944	25.093	1.449	9	0.161
256	6052.798	23.644	13.984	4	3.496
1280	17529.760	9.660			

Each quadrat is divisible into 64 equal block units.

APPENDIX 1

Field study data (Lundin Links) is bound in separate data volume.

Table 7. Means and correlation matrix of characters of control strip plants measured in June 1969.

		Leaf no.	Leaf width (mm)	Leaf length (mm)	Inflo. no.	Scape length (mm)	Spike length (mm)	Growth habit (1-5)
Means		4.4	7.1	46.4	2.6	113.8	8.7	3.6
Leaf no.	(corr. coeff)		0.55	0.31	0.20	0.21	0.40	-0.12
	(significance)		***	***	*	*	***	
Leaf width				0.55	0.17	0.42	0.67	0.07
				***		***	***	
Leaf length					0.31	0.72	0.60	0.53
					**	***	***	***
Inflo. no.						0.13	0.22	0.05
							*	
Scape length							0.65	0.57
							***	***
Spike length								0.23
								*

Table 8. Correlation between height of vegetation and characters of plants in field.

	Leaf no.	Leaf width	Leaf length	Inflo. no.	Scape length	Spike length	Growth habit
Ht. veg. June 1969	-0.02	0.27	0.60	-0.11	0.51	0.29	0.31
		*	***		**	*	***
Mean ht. veg.	-0.00	0.08	0.39	-0.00	0.25	0.25	0.52
June 1969 - Sept. 1971			***		**	**	***

N.B. See Appendix 1, p 201 for interpretation of correlation matrices.

Note on statistical methods

Interpretation of correlation matrices

The levels of significance on the correlation matrices represent significance of the correlation coefficients taken singly. Clearly some correlations may be significant purely by 'chance' since many comparisons are being tested. Clearly it would be desirable to have a simultaneous test procedure (such as Duncan's Multiple Range Test) to pick out only those correlations that are sufficiently different from zero to be deemed significant in a multiple comparison situation. However, I have been unable to discover such a test procedure. This is particularly aggravated in the case of a correlation matrix since each test is not independent of other tests but correlated with one or more of them: simply adjusting the significance criterion is also invalid.

For this reason much of the interpretation of the correlations is on an intuitive or semi-quantitative basis, as in tables 10 and 11, p 204. The correlations significant at the .05 level were given a score of 1, those at the .01 level a score of 2 and those significant at the .001 level were given a score of 3. A correlation score was then assigned to each morphological character. No attempt is made in the text to discuss specific correlations in detail since individual values may be spuriously significant. Only correlations that are highly significant or that appear consistently in a number of treatments have been emphasized.

Multiple regression methods

Multiple regression specifies the relationship of one dependent variable to a set of independent variables.

$(Y = c + a_1x_1 + a_2x_2 + ...a_nx_n)$

From the multiple regression the multiple correlation can be calculated: the multiple correlation of a dependent variable with a set of independent variables is defined as the correlation of the dependent variable (Y) with the value of that variable predicted by the multiple regression equation using observed values of the independent variables (x_1 to x_n). This multiple correlation coefficient (R) is related to the amount of variation in a variable (Y) that can be estimated from a number of independent variables (x_1 to x_n). More precisely the actual proportion of variation that can be accounted for in this way is estimated by R^2, the squared multiple correlation.

The computer technique (R^2 maximum improvement) used in the multiple regression analysis involved finding the best one-variable model (i.e. model which maximised R^2), the best two-variable models, and so on.

The eventual multiple regression equation, and hence the variables associated with the dependent variable, was chosen using the criterion that the regression be the best fit (P = a minimum) and that it be a significant fit at the $P < 0.05$ level.

APPENDIX 1

Table 9. Correlation matrices of characters of plants in the gardens and greenhouse.

Gardens 1970

		Leaf no.	Leaf width	Leaf length	Inflo. no.	Scape length	Spike length	Growth habit
June 1970 correlation matrix								
Leaf number	(corr. coeff.)		-0.15	0.22	0.18	0.23	0.17	0.01
	(significance)			*		**		
Leaf width		-0.08		0.28	-0.02	0.34	0.34	-0.12
				*		***		
Leaf length		-0.09	0.28		0.42	0.61	0.58	0.45
			*		***	***	***	***
Inflo. no.		0.16	-0.02	0.07		0.35	0.40	0.07
						**	***	
Scape length		-0.09	0.20	0.59	-0.07		0.69	0.23
			*	***			***	***
Spike length		-0.10	0.48	0.39	-0.04	0.46		0.21
			***	***		***		**
Growth habit		-0.05	0.02	0.46	-0.13	0.36	0.17	
				***		***	**	
September 1970 correlation matrix								

Greenhouse June 1970

Clone date 1 correlation matrix								
Leaf number	(corr. coeff.)		-0.16	-0.29	0.14	-0.16	-0.07	-0.21
	(significance)		*	***	**	*		***
Leaf width		0.55		0.08	0.13	0.12	0.30	-0.12
		***					***	
Leaf length		0.33	0.46		-0.41	0.23	0.08	0.44
		***	***		***	**		***
Inflo. no.		0.42	0.29	0.30		0.27	0.34	-0.22
		***	***	***		***	***	**
Scape length		0.28	0.21	0.37	0.71		0.72	0.20
		***	**	***	***		***	**
Spike length		0.34	0.25	0.34	0.68	0.89		0.14
		***	***	***	***	***		*
Growth habit		-0.25	-0.10	0.28	-0.05	0.23	0.20	
		***	**	***		**	**	
Clone date 2 correlation matrix								

Greenhouse September 1970

Clone date 1 correlation matrix								
Leaf number	(corr. coeff.)		-0.19	-0.12	-0.08	-0.37	-0.31	-0.11
	(significance)		**			***	***	
Leaf width		-0.13		0.26	-0.08	0.06	0.23	0.06
				***			***	
Leaf length		-0.27	0.37		-0.33	0.52	0.12	0.41
		***	***		***	***		***
Inflo. no.		0.12	-0.22	-0.27		-0.14	-0.19	-0.20
			**	***			*	*
Scape length		-0.50	0.09	0.50	-0.19		0.36	0.26
		***		***	*		***	***
Spike length		-0.28	0.23	0.27	-0.19	0.49		0.16
		***	**	***	*	***		
Growth habit		-0.31	0.03	0.46	-0.12	0.40	0.14	
		***		***	**	***		
Clone date 2 correlation matrix								

Table 10. Correlation 'scores' of plants in garden, greenhouse and field.

(Appendix 1, p 201 for information on correlation scores.)

Character	Leaf width	Leaf length	Inflo. no.	Scape length	Spike length	Growth habit
Leaf no.	- -1 3 - -2 - 3	1 -3 3 - - -3 3	- -1 3 - - - 1	2 -1 3 - -3 -3 1	- - -3 - -3 -3 3	1 -2 -3 - - -3 1
Leaf width		1 - 3 1 3 3 3	- - 3 - - -2 -	3 - 2 1 - - 3	- 3 3 3 2 2 3	- - -2 - - - -
Leaf length			3 -3 3 - -3 -3 2	3 2 3 3 3 3 3	3 - 3 3 - 3 3	3 3 3 3 3 3 3
Inflo. no.				3 3 3 - - -1 -	3 3 3 - -1 -1 1	- -2 - - -1 -2 -
Scape length					3 3 3 3 3 3 3	3 2 2 3 3 3 3
Spike length						2 1 2 2 - - 1

Key: 'Scores': 3 - $P < 0.001$
2 - $P < 0.01$
1 - $P < 0.05$
- - not significant

A	B	C
D	E	F
	G	

A - garden, June 1970
B - greenhouse, clone date 1, June 1970
C - " " " 2, " "
D - garden, September 1970
E - greenhouse, clone date 1, September 1970
F - " " " 2, " "
G - field, June 1969

Table 11. Comparison of field correlation scores with the total correlation scores.

Character	Leaf width	Leaf length	Inflo. no.	Scape length	Spike length	Growth habit
Leaf no.	3 (3)	3 (1)	1 (3)	1 (-1)	3 (0)	1 (-6)
Leaf width		3 (14)	0 (1)	3 (9)	3 (16)	-2 (0)
Leaf length			2 (-1)	3 (20)	3 (15)	3 (21)
Inflo. no.				0 (8)	1 (8)	0 (-5)
Scape length					3 (21)	3 (19)
Spike length						1 (8)

Key: 1st. figure = score field correlation
2nd. figure = total of all correlation scores (bracketed)

Table 12. Correlations of vegetation height with characters of plants in garden, greenhouse and field.

Environment	Leaf no.	Leaf width	Leaf length	Inflo. no.	Scape length	Spike length	Growth habit
Garden. June	0.18	-0.15	0.06	0.09	-0.12	-0.10	0.19
Sept	-0.23	0.13	0.22	-0.09	0.06	0.02	0.19
Greenhouse.							
June Clone date 1	0.07	-0.06	0.06	-0.16	-0.10	-0.15	0.11
" Clone date 2	0.18	0.13	0.05	(-0.00)	-0.11	-0.06	-0.01
Sept. Clone date 1	0.22	0.03	0.01	-0.13	-0.18	-0.15	0.11
" Clone date 2	0.11	-0.02	0.00	-0.03	-0.11	0.07	-0.02
Field	-0.00	0.08	0.39	-0.00	0.25	0.25	0.52

Table 13. Significance levels of correlations of vegetation height with characters of plants in garden, greenhouse and field.

Environment	Leaf no.	Leaf width	Leaf length	Inflo. no.	Scape length	Spike length	Growth habit
Garden. June	0 *				0		0 *
Sept.	0 *		0 *				*
Greenhouse.							
June Clone date 1	*			0 *		0 *	0
" Clone date 2	0 *				0		
Sept Clone date 1	0 **	0		0	0 *	0 *	0
" Clone date 2	0				0	0	
Field			***		***	**	***

Key: 0 $P < 0.05$ - Multiple regression (see Appendix 1, p 202).
* $P < 0.05$ - Correlation
** $P < 0.01$ - "
*** $P < 0.001$ - "

Table 14 Correlation matrix of Phenotypes in field with phenotypes under standard conditions

Lundin Links	Standard Conditions: Leaf No June	Leaf No Sept.	Leaf width June	Leaf width Sept	Leaf length June	Leaf length Sept	Inflo. No. June	Inflo. No. Sept	Scape length June	Scape length Sept	Spike length June	Spike length Sept	Growth habit June	Growth habit Sept	
Leaf No	0.10	-0.21	-0.01	0.08	-0.11	-0.02	-0.14	-0.13	-0.12	-0.05	0.03	0.06	-0.02	-0.16	A
	0.25	0.17	-0.11	-0.18	-0.06	0.07	0.02	-0.07	-0.00	0.03	0.05	-0.03	0.06	0.04	B
	-0.04	-0.07	-0.12	-0.07	-0.09	-0.14	-0.18	-0.09	-0.11	-0.15	0.10	0.04	0.03	0.05	C
Leaf width	0.05	-0.15	0.02	0.09	0.09	0.05	-0.16	-0.20	-0.06	-0.05	-0.08	-0.08	-0.02	0.04	
	0.16	0.03	0.01	-0.02	-0.05	0.11	-0.09	-0.12	-0.09	0.04	0.07	0.06	0.17	0.17	
	-0.09	-0.10	-0.16	-0.03	-0.16	0.06	-0.17	-0.12	-0.20	-0.01	-0.02	0.04	0.12	0.10	
Leaf length	0.09	-0.04	-0.07	0.24	0.26	0.27	0.02	-0.07	-0.01	0.11	0.01	0.12	0.37	0.32	
	-0.08	-0.11	0.04	0.05	0.05	0.18	-0.21	-0.26	-0.04	0.03	-0.02	-0.04	0.16	0.22	
	0.11	0.03	0.03	-0.05	0.03	0.22	0.08	0.00	0.07	0.16	-0.09	0.09	0.17	0.26	
Inflo No.	0.12	-0.27	-0.07	0.44	-0.18	-0.20	-0.21	0.04	-0.11	-0.09	0.04	0.26	-0.17	-0.21	
	0.32	0.23	0.15	-0.13	-0.01	-0.11	0.19	0.05	-0.13	-0.16	0.21	0.11	-0.32	-0.22	
	-0.07	0.12	-0.10	-0.00	-0.20	-0.34	-0.03	0.04	0.11	-0.39	0.44	-0.02	-0.06	-0.05	
Scape Length	0.23	-0.17	-0.19	0.20	0.32	0.28	-0.04	-0.08	0.06	0.17	-0.05	0.06	0.53	0.56	
	-0.04	-0.09	0.32	0.17	0.20	0.19	-0.35	-0.28	0.01	0.05	0.13	0.18	0.20	0.28	
	-0.22	-0.31	-0.19	0.03	0.08	0.48	-0.18	-0.13	-0.16	0.20	-0.26	-0.00	0.43	0.52	
Spike Length	0.16	-0.32	-0.19	0.42	0.15	0.16	-0.08	-0.06	-0.00	0.05	0.03	0.17	0.31	0.29	
	0.30	0.19	0.10	-0.15	0.15	0.19	-0.11	-0.06	0.04	0.08	0.29	0.34	0.09	0.07	
	-0.18	-0.24	-0.15	0.05	0.11	0.30	-0.10	0.01	-0.06	-0.12	-0.05	0.11	0.30	0.35	
Growth habit	0.03	0.07	0.01	0.07	0.18	0.23	0.09	0.05	-0.05	0.02	0.08	0.05	0.40	0.37	
	0.02	-0.05	0.03	0.05	0.09	-0.12	-0.04	-0.02	-0.07	-0.09	0.05	-0.05	0.11	0.05	
	-0.02	-0.05	0.03	0.05	0.14	0.23	0.01	0.02	-0.05	-0.01	-0.05	0.01	0.13	0.26	
Mean vegetation height	0.13	-0.14	-0.14	0.13	0.16	0.27	0.06	-0.04	-0.06	0.11	-0.07	-0.26	0.16	0.19	
	0.16	0.23	-0.01	0.07	0.01	0.12	-0.23	-0.20	-0.14	-0.08	-0.13	-0.10	0.05	0.12	
	0.20	-0.09	0.10	-0.14	0.08	0.15	-0.08	-0.02	-0.13	0.08	0.07	0.03	0.04	0.26	
Variance in vegetation height	0.21	-0.07	-0.06	0.26	0.15	0.23	0.10	0.03	-0.07	0.01	0.01	0.07	0.01	0.03	
	0.11	0.20	-0.09	0.03	0.00	0.14	-0.26	-0.26	-0.10	-0.04	-0.17	-0.14	-0.09	0.05	
	0.32	0.10	0.06	-0.24	-0.00	0.03	0.09	0.02	-0.16	-0.04	0.05	-0.03	-0.05	0.14	

A Gardens
B Greenhouse 1
C Greenhouse 2

Statistical significance of correlation coefficients:

If correlation coefficient $\geq$ 0.32, $P < 0.001$

If correlation coefficient $\geq$ 0.25, $P < 0.01$

If correlation coefficient $\geq$ 0.20, $P < 0.05$

APPENDIX 1

Table 15 a) Levels of significance of correlations of field characters with same character under standard conditions

STANDARD CONDITIONS

FIELD	Leaf No. June	Leaf No. Sept	Leaf width June	Leaf width Sept	Leaf length June	Leaf length Sept	Inflo. No. June	Inflo. No. Sept	Scape length June	Scape length Sept	Spike length June	Spike length Sept	Growth habit June	Growth habit Sept
Leaf No.	- ••• - - 0 -	-• • - 0 0 0		-• 0 	 0 	 0 	 	 0 	 0 	 0 	 0 	 	 0 0	 0
Leaf width	- • - - 0 -	- - - - - -	-• 0 	 	-• 0 0 	 	 0 	 0 	 0 	 	 	 	• 0 0	•
Leaf length	 	- - - - 0 -	 	•• 0 	•• 0 	•• • •• 	 -•• 0 	 -•• 0 	 0 	 	 0	 	••• • • 0 	••• •• ••• 0 0 0
Inflo. No.	•• 0 0	 0 0 	 	•• 0 	 -• 0	 -•• 0 	 	 0 	 0 0	 -•• 0	 • 0 0	 0 0	-•• 0	 0
Scape Length	 -• 0 0 0	 -•• 	 •• 0 	 0 	• 	 ••• 0 	 -•• 0 	 -• 0 	 	 	 0 	 	••• ••• 0 0	••• • ••• 0 0
Spike Length	 • 0 	-• -• 0 0 0	 0 	•• 0 	 	 0 	 0 	 	 0	 	• 0 0	 	• •• 0 	• •• 0
Growth habit	 0 	 	 	 	• • 0	•• • 0 	 0 	 0 	 0 0	 0 	 0	 	••• 0 	••• •• 0
Mean height of vegetation	 •• 0	•• 0 	 	-• 0 0	 0 	•• • 0 	-•• 0 	-•• 0 	 0	 	 0	 	 0 	 •• 0 0
Variance in height of vegetation	 ••• 0 0 0	•• 0 	 	•• -•• 0 0	 0 	• 0 	-••• 0 	-•• 0 	 0 0	 	-• 	 0 	 0 	 0

Table 15 b) Correlation 'scores' of above table (see 'notes on statistical methods')

STANDARD CONDITIONS

FIELD	Leaf No	Leaf width	Leaf length	Inflo. No.	Scape length	Spike length	Growth habit
Leaf No	7	-2	2	1	2	1	3
Leaf width	2	-2	-3	2	1	-	4
Leaf length	1	3	8	-6	1	1	17
Inflo No	5	3	-5	1	-5	5	-5
Scape length	-6	4	5	-5	-	1	18
Spike length	-4	4	1	1	1	5	9
Growth habit	1	-	7	1	3	1	12
Height vegn.	6	-3	5	-6	1	1	5
Variance vegn. Height	10	3	3	-7	3	-2	2

- = Gardens
- = G/House 1
- = G/House 2
- = Gardens
- = G/House 1
- = G/House 2

Corrn. - Multiple Regression

APPENDIX 1 Table 16. Flowering time data (collated for different phenotypes as classified by growth habit

Date	Prostrate habit							Intermediate habit							Erect habit						
	Flowering stage							Flowering stage							Flowering stage						
	1	2	3	4	5	6	Mean	1	2	3	4	5	6	Mean	1	2	3	4	5	6	Mean
4.5.70	20	2	0	0	0	0	1.09	5	79	3	3	0	0	2.04	4	35	7	1	0	0	2.11
11.5.70	23	66	3	0	0	0	1.38	64	49	21	0	0	0	1.68	14	21	17	0	0	0	2.06
18.5.70	31	8	5	1	0	0	1.47	51	53	42	7	8	0	2.18	6	16	21	3	6	0	2.75
25.5.70	17	8	15	1	5	0	2.33	20	45	41	15	29	8	3.08	3	9	21	16	12	3	3.33
1.6.70	21	0	7	2	5	9	2.93	17	10	33	14	35	45	4.14	22	1	9	8	23	10	4.49
8.6.70	6	0	3	0	4	18	4.61	5	5	2	2	28	90	5.37	2	1	1	0	12	36	5.44
8.5.70	12	1	0	0	0	0	1.08	89	15	1	0	0	0	1.16	132	48	0	0	0	0	1.27
15.5.70	6	10	1	0	0	0	1.71	107	74	10	0	0	0	1.49	157	158	20	0	0	0	1.59
22.5.70	10	15	6	0	0	0	1.87	98	140	72	4	0	0	1.94	143	243	138	5	1	0	2.02
29.5.70	11	17	18	6	0	0	2.37	124	181	194	45	11	0	2.35	158	310	292	82	31	0	2.49
5.6.70	16	30	30	10	10	0	2.67	169	251	322	113	96	8	2.73	219	386	413	180	196	12	2.85
19.6.70	17	39	62	28	38	17	3.41	159	395	494	238	479	275	3.64	207	550	706	327	707	476	3.74
8.5.70	6	7	1	0	2	3	2.56	35	33	36	7	35	15	3.06	51	86	43	12	93	23	3.25
15.5.70	4	10	1	3	2	3	2.91	58	60	25	21	51	40	3.26	63	81	50	36	98	64	3.55
22.5.70	4	8	7	5	11	9	3.86	65	89	66	23	90	89	3.59	84	80	71	46	156	143	3.93
29.5.70	6	8	2	2	16	15	4.12	76	75	78	48	67	182	3.95	59	98	65	43	116	293	4.39
5.6.70	4	8	7	2	7	25	4.42	54	76	67	42	98	235	4.33	52	76	89	36	87	369	4.85
12.6.70	7	8	5	2	6	36	4.56	63	104	61	27	104	341	4.47	84	104	63	42	113	454	4.58

Table 17. Correlation of spike length and seed number

Spike length (mm)	Number of seeds
4	0
5	3, 6, 6, 0, 2, 2, 2, 2, 3, 0, 0, 0, 6, 7, 6
6	0, 3, 5
7	1, 0, 5, 2, 9, 6, 0, 5, 5, 10, 8, 8, 10, 8, 8, 10, 10
8	7, 7, 0, 12, 7, 4, 13, 10, 9, 12, 15, 4, 6, 9, 8
9	8, 11, 12, 20, 5, 10, 12, 9, 10, 11, 12
10	12, 13, 17, 6, 11, 10, 25, 13, 14, 6, 6, 20, 21, 24, 13, 8, 9
11	6, 3, 9
12	12, 11, 17, 15
13	24, 21, 15
15	15, 19, 20, 31, 37, 22, 17, 17
17	41, 17
21	42

Regression: $a = 2.06 \pm 0.15$

$b = -8.122$

Correlation coefficient = 0.80, $P < 0.01$

Table 18. Survivorship at Lundin Links

a) Control strip

Stage of duration (each of 3 months)										Plants already established or arriving in:-
1	2	3	4	5	6	7	8	9	10	
194	164	149	115	106	99	98	96	94	80	June 1969
	180	162	127	118	110	109	106	103	90	September 1969
		196	149	136	125	123	120	117	101	December 1969
			183	163	147	145	140	135	112	March 1970
				197	177	175	169	164	140	June 1970
					207	202	195	190	159	September 1970

b) Control strip - Survivorship of new arrivals

Stage of duration (each of 3 months)									Plants arriving in:-
2	3	4	5	6	7	8	9	10	
30	26	17	16	13	13	11	10	9	September 1969
	15	10	7	6	6	6	6	5	December 1969
		41	34	30	30	28	26	19	March 1970
			31	29	29	28	28	27	June 1970
				29	28	25	25	18	September 1970

c) Experimental strip - Survivorship of new arrivals

Stage of duration (each of 3 months)									Plants arriving in:-
2	3	4	5	6	7	8	9	10	
54	52	51	43	40	40	40	38	36	September 1969
	64	61	51	48	48	48	45	42	December 1969
		69	58	54	54	54	51	46	March 1970
			69	64	61	61	58	52	June 1970
				90	87	82	76	66	September 1970

APPENDIX 2.

Drosophila melanogaster data

Food medium

The medium described by Mittler & Bennett (1962) was used because it is easy to prepare, easy to dispense and is more homogeneous than many other media. Nipagin was used instead of propionic acid as a fungicide. The recipe is as follows:

1000ml distilled water

54g sucrose

32g dried active baking yeast (Allinson Ltd., 210/214 Cambridge Heath Rd., London E.2)

19g agar (David Gelatine Ltd., Warwick)

26ml nipagin solution - 10g nipagin in 100ml 96% ethanol.

The water was heated to boiling point and then all the ingredients except the nipagin were added. The mixture was simmered for 20 min. with occasional stirring until the level evaporated down to a fixed graduation mark. This was to ensure standardisation of the moisture content of the medium. It was then removed from the heat and allowed to cool to 60°C when the nipagin was added. The medium was then maintained at approximately 60°C until dispensing. 12 hours before the flies were introduced, the medium was seeded with live yeast suspension (5g in 100ml distilled water).

The flies

Drosophila melanogaster (stock CB7) were obtained from the University College of North Wales, Bangor. This stock was descended from one inseminated female caught near Bangor in 1965 and subsequently reared in bottles in the laboratory on the medium described on p 212. Because of the relatively short history of laboratory culture, it was hoped that this stock would be genetically very variable. Counts of sternopleural chaeta numbers by Antonovics (pers. comm.) showed this character to have a significant heritability. In order to minimise any loss of variability due to inbreeding, F_1 progeny from crosses between flies from each of the two stock bottles kept in the laboratory were used to set up the experiments.

At 25°C in an anhydric incubator, the life cycle was as follows:

Eggs laid

Pupation - day 6 after eggs laid

First emergence - day 10 after eggs laid

Peak emergence - day 18 after eggs laid.

All experimental stocks were kept at 25°C and arranged in random order within the incubator.

Anaesthetic

Ether, administered in an etheriser, was used as the anaesthetic for the flies. Since ether is known to adversely affect egg-laying (Thoday, pers. comm.), the possibility of the use of carbon dioxide (CO_2) was investigated. CO_2 was found to be impracticable, however, as the flies remained inert only as long as the CO_2 diffused around them. The quick recovery of the flies was a problem in the main experiment when CO_2 could not be supplied to the flies being weighed and, for this reason, it was not used.

Table 1. Results of experiment to investigate toxicity of peppermint to D. melanogaster.

Conc^{n} ppt. (%)	No. offspring day 23 Replicates 1	2	3	4	5	Total	Mean	s.e.	(%) emergence
0.00	138	161	157	167	161	784	156.8	5.4	100.0
0.03	161	138	166	158	148	771	153.9	5.0	98.3
0.04	114	170	175	165	171	793	158.6	11.4	101.1
0.05	162	181	155	142	152	792	158.4	6.5	101.0
0.07	186	125	165	190	155	821	164.2	11.8	104.7
0.10	187	124	165	163	139	778	155.5	11.0	99.2
0.30	37	169	130	174	141	651	130.2	24.7	83.0
0.50	88	130	135	124	44	521	104.2	17.2	66.5
0.70	60	108	55	44	101	368	73.6	12.9	46.9
0.90	22	52	66	16	8	164	32.8	11.1	20.9
1.00	0	0	0	0	0	0	0.0	0.0	0.0

Correlation of number of offspring on day 23 with peppermint concentration:

$r = -0.977$ (significant at 0.001% level)

Table 2. Mortality of parents on day 6

Pp$^{t.}$ Conc$^{n.}$(%)	No. dead / 6 adults (4♀♀ & 2♂♂)								dead males			dead females			
	Replicates					Total	Ave / vial	s.e.	% mortality	total	ave	%	total	ave	weighted ave	%
	1	2	3	4	5											
0.00	0	0	4	0	3	7	1.4	± 0.9	23	3	0.6	30	4	0.8	0.4	20
0.03	0	2	1	2	2	7	1.4	± 0.4	23	3	0.6	30	4	0.8	0.4	20
0.04	2	0	2	0	0	4	0.8	± 0.5	13	3	0.6	30	1	0.2	0.1	5
0.05	0	0	0	0	0	0	0.0	± 0.0	0	0	0.0	0	0	0.0	0.0	0
0.07	0	0	0	0	0	0	0.0	± 0.0	0	0	0.0	0	0	0.0	0.0	0
0.10	3	2	2	2	1	10	2.0	± 0.3	33	5	1.0	50	5	1.0	0.5	25
0.03	0	2	3	3	3	11	2.2	± 0.6	37	7	1.4	70	4	0.8	0.4	20
0.50	2	0	3	3	5	13	2.6	± 0.8	43	7	1.4	70	6	1.2	0.7	35
0.70	2	2	5	6	4	19	3.8	± 0.8	63	9	1.8	90	10	2.0	1.0	50
0.90	4	4	3	5	6	22	4.4	± 0.5	73	10	2.0	100	12	2.4	1.2	60
1.00	6	6	6	6	6	30	6.0	± 0.0	100	10	2.0	100	20	4.0	2.0	100

Table 3. Survival of different stages of D. melanogaster on peppermint

Peppt. conc n. (% essence)	Rep	Productivity: Eggs laid by 7 females: nos.	ave.	s.e.	ave %	% survival of 20 transferred individuals: Egg to pupa %	ave	s.e.	Larva to pupa %	ave	s.e.	Pupa to adult %	ave	s.e.
0	1	202	196.3	± 7.1	100	40	56	±17.5	90	98	± 2.5	95	99	± 1.3
	2	189				90			100			100		
	3	181				80			100			100		
	4	213				15			100			100		
0.3	1	97	120.3	±21.4	61	55	83	± 9.7	100	100	± 0	100	100	± 0
	2	-				100			100			100		
	3	163				85			100			100		
	4	101				90			100			100		
0.5	1	58	69.5	±16.4	35	50	80	±10.8	100	98	± 2.5	100	98	± 1.4
	2	29				90			100			95		
	3	103				80			100			100		
	4	88				100			90			95		
0.7	1	149	120.3	±10.4	61	75	80	± 8.4	95	98	± 1.4	90	98	± 2.5
	2	112				60			100			100		
	3	120				100			95			100		
	4	100				85			100			100		
0.9	1	-	42.5	±27.4	22	80	79	± 2.4	80	95	± 5.0	100	99	± 1.3
	2	115				85			100			100		
	3	-				75			100			95		
	4	55				75			100			100		

Table 4. Response of families to peppermint

a) Yield expressed as number of emergent progeny

	Peppermint concentration					
	0.0% essence		0.6% essence		0.9% essence	
Family	no. progeny	ave s.e.	no. progeny	ave s.e.	no. progeny	ave s.e.
1	142, 60, 27	76.3 ± 34.0	94, 30, 28	50.7 ± 22.0	35, 59	47.0 ± 12.0
2	108, 37	72.5 ± 36.0	34, 4, 34	24.0 ± 10.0	13, 3	8.0 ± 5.0
3	171, 147, 127, 176, 175, 160	159.3 ± 7.9	82, 106, 101, 38, 151, 155	105.5 ± 17.9	72, 52, 64, 43, 75, 95	66.8 ± 7.5
4	172, 159, 127	152.7 ± 13.4	57, 96, 122	91.7 ± 18.9	22, 56	39.0 ± 17.0
5	72, 161, 109, 129	117.8 ± 18.6	30, 155, 109, 63	89.3 ± 27.3	58, 52, 98	69.3 ± 14.4
6	118, 3	60.5 ± 57.5	24, 24	24.0 ± 0.0	62, 29	45.5 ± 16.5
7	189, 175, 163, 163	172.5 ± 6.2	112, 104, 123, 92	107.8 ± 6.5	81, 82, 63 26	63.0 ± 13.1

Analysis of variance - from table 4a data

Source of variance	dF	Sum Sq.	Mean Sum Sq.	F ratio
Families	6	63876.02	10646.00	7.23 (P<0.01)
Concentrations	2	52329.01	26164.51	18.94 (P<0.001)
Family x concentration	12	29185.04	2432.09	1.97 (NS)
Within families	49	60582.92	1236.38	1471.6
Total	69	205972.99		

b) Yield expressed as proportion of yield on 0 peppermint concentration

	Peppermint concentration			
	0.6% essence		0.9% essence	
Family	Prop^{n} progeny	Ave s.e.	Prop^{n} progeny	Ave s.e.
1	1.23, 0.39, 0.37	0.66 ± 0.20	0.46, 0.77	0.62 ± 0.19
2	0.47, 0.06, 0.47	0.33 ± 0.20	0.18, 0.04	0.11 ± 0.19
3	0.51, 0.67, 0.63, 0.24, 0.95, 0.97	0.66 ± 0.14	0.45, 0.33, 0.40, 0.27, 0.47, 0.60	0.42 ± 0.09
4	0.37, 0.63, 0.80	0.60 ± 0.20	0.14, 0.37	0.25 ± 0.19
5	0.25, 1.32, 0.93, 0.53	0.76 ± 0.18	0.49, 0.44, 0.83	0.59 ± 0.13
6	0.40, 0.38	0.39 ± 0.30	1.03, 0.48	0.75 ± 0.19
7	0.65, 0.60, 0.71, 0.53	0.62 ± 0.18	0.47, 0.48, 0.37, 0.15	0.37 ± 0.11

Analysis of variance - from table 4b data

Source of variation	Peppermint concentration	F ratio
Between families	0.6% essence	0.770 (NS)
" "	0.9% essence	9.55 (P < 0.01)

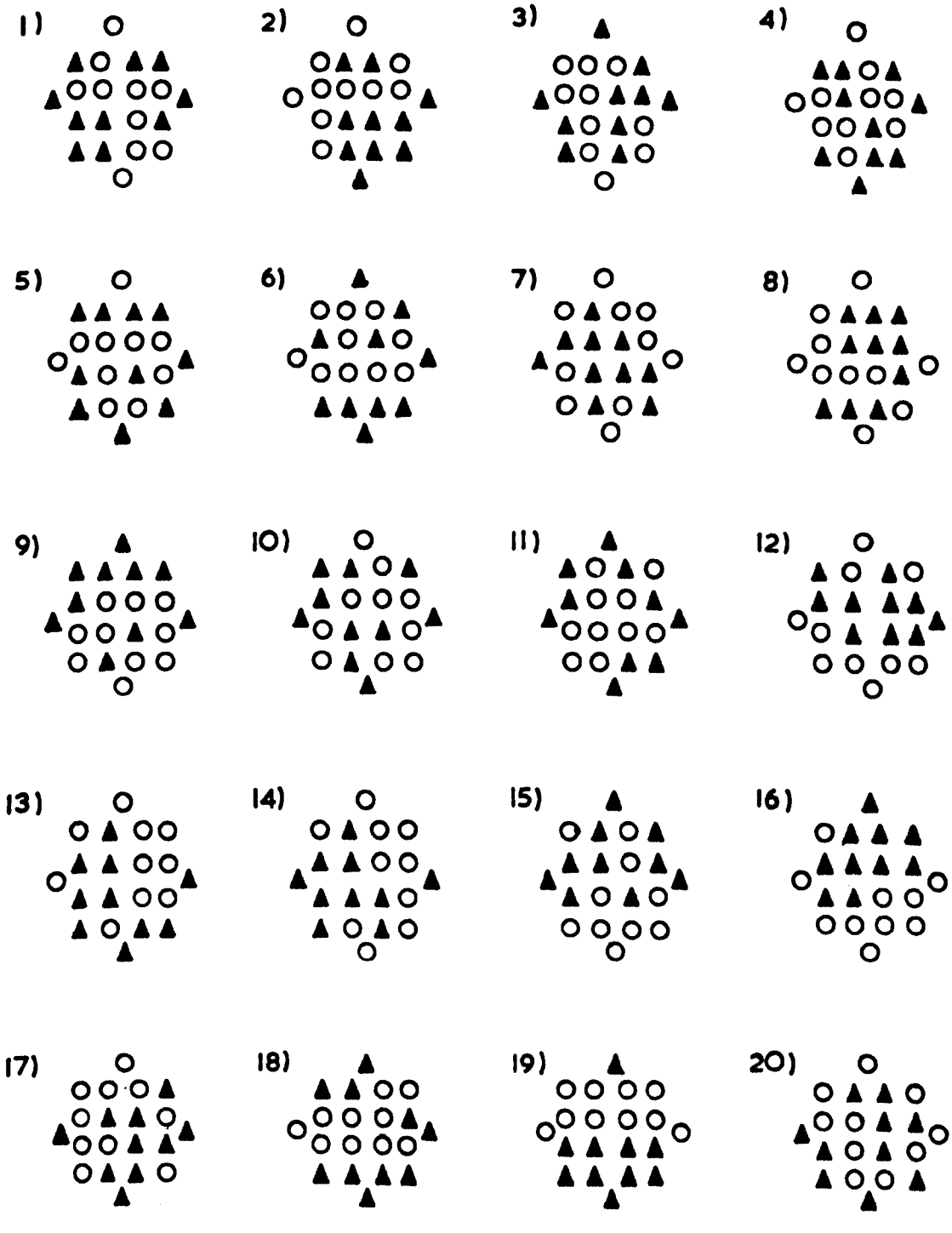
Containers

For the main experiments, 250ml Pyrex glass beakers were used for the population cages. To keep fungal infection to a minimum, each beaker was sterilised by autoclaving at a pressure of 15lb per sq in for 20min before use. An expanded polystyrene disc, containing 20 holes bored by a red hot wire and swabbed with alcohol was pushed into the beaker (fig. 24, p 121). 20 autoclaved glass tubes (35 x 8mm) were inserted into the holes and food medium at 60°C was dispensed into the tubes with a pipette. The food in each beaker totalled 13.2ml. Each tube of food was then seeded with 1 drop of live yeast suspension (5g in 100ml distilled water). The beaker, stoppered with a sterile cotton wool bung, was allowed to stand overnight to allow any condensation to evaporate. The anaesthetised flies were then introduced and the beaker was covered by clear polythene sheeting, perforated to allow aeration.

APPENDIX 2

Randomised heterogeneous systems

10 N : 10 P tubes



○ tube of normal medium

▲ tube of peppermint medium

APPENDIX 2

Randomised heterogeneous systems

15 N : 5 P or 5 N : 15 P tubes

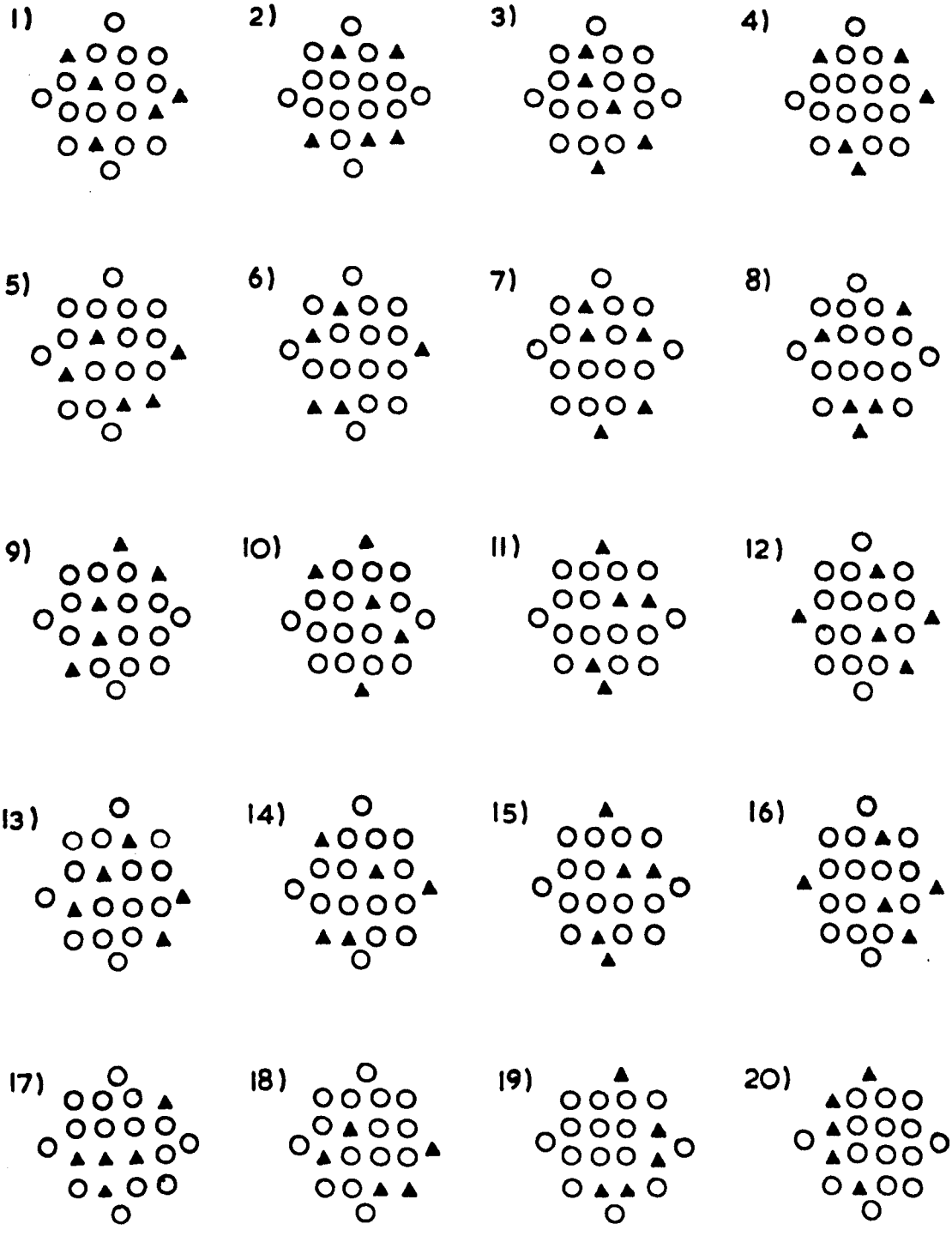


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Table 5. Peppermint experiment - yield over 18 generations expressed as number of emergent flies.

Treatment	Rep	Generation 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
N control	A	101	246	187	270	275	177	198	268	309	217	328	283	378	323	301	204	179	239
	B	109	274	188	286	259	218	244	276	272	211	239	292	339	350	276	244	253	262
	C	201	215	129	542	304	202	192	268	279	211	307	282	371	364	294	238	293	298
	Ave.	137	245	168	366	279	199	211	271	286	213	291	285	362	346	288	229	241	266
	s.e.	± 32	± 17	± 20	± 88	± 13	± 12	± 16	± 3	± 11	± 2	± 27	± 3	± 12	± 12	± 7	± 12	± 33	± 17
M control	A	56	170	254	286	294	242	242	295	259	193	298	303	309	307	249	295	376	245
	B	124	221	258	290	358	248	245	251	258	199	262	306	332	333	254	260	294	273
	C	128	191	185	266	284	240	254	280	272	268	327	241	250	331	252	228	278	265
	Ave.	103	194	232	280	312	243	246	275	263	220	296	283	297	324	252	261	316	261
	s.e.	± 23	± 15	± 24	± 7	± 24	± 2	± 4	± 13	± 5	± 24	± 19	± 21	± 24	± 8	± 1	± 19	± 30	± 8
P control	A	69	212	228	219	280	178	194	265	281	193	235	237	340	285	182	131	264	210
	B	91	157	155	235	233	128	158	257	261	143	232	233	306	228	124	110	293	198
	C	94	254	165	281	264	216	164	270	268	227	290	293	334	287	202	202	285	177
	Ave.	84	207	182	242	259	174	172	264	270	188	252	254	326	270	169	147	278	195
	s.e.	± 8	± 28	± 23	± 18	± 14	± 25	± 11	± 4	± 6	± 24	± 19	± 19	± 10	± 19	± 23	± 28	± 9	± 10
H popul^{n}.	A	95	147	179	293	Separated tubes	222	378	318	352	327	264	266	304	278	232	211	221	187
	B	101	203	199	293		181	193	265	284	283	395	344	355	302	260	236	259	302
	C	98	225	215	274		204	170	293	292	192	286	282	334	282	271	247	169	210
	Ave.	98	192	198	287		202	247	292	310	267	315	297	331	287	254	231	216	233
	s.e.	± 2	± 23	± 10	± 6		± 12	± 66	± 15	± 21	± 40	± 41	± 24	± 15	± 7	± 12	± 11	± 26	± 35

Table 6. Peppermint experiment - average weight per fly over 18 generations of selection

Treatment	Rep	Generation 3	4	5	6	7	8	9	10	11	13	14	15	16	17	18
N control	A	.706	.756	.742	.785	.758	.817	.819	.747	.762	.709	.854	.811	.690	.854	.800
	B	.723	.790	.745	.702	.754	.779	.757	.734	.741	.720	.846	.761	.735	.840	.802
	C	.713	.509	.740	.817	.760	.813	.789	.754	.765	.730	.813	.752	.706	.841	.782
	Ave.	.714	.684	.743	.767	.757	.803	.788	.745	.758	.719	.837	.774	.710	.845	.795
	s.e.	±.009	±.153	±.003	±.059	±.003	±.021	±.031	±.010	±.014	±.011	±.022	±.032	±.023	±.008	±.011
M control	A	.866	.815	.752	.826	.614	.790	.826	.798	.812	.880	.785	.839	.722	.746	.941
	B	.845	.828	.757	.788	.785	.848	.868	.819	.794	.081	.835	.732	.773	.766	.846
	C	.984	.763	.757	.724	.750	.796	.830	.787	.844	.780	.831	.734	.773	.774	.851
	Ave.	.898	.804	.755	.780	.717	.810	.841	.799	.819	.824	.819	.768	.756	.762	.882
	s.e.	±.075	±.034	±.003	±.052	±.091	±.026	±.023	±.016	±.025	±.053	±.029	±.061	±.029	±.014	±.054
P control	A	.868	.877	.804	.831	.794	.838	.811	.865	.864	.812	.839	.846	.761	.790	.913
	B	.871	.749	.824	.844	.785	.798	.835	.839	.815	.801	.851	.798	.709	.755	.862
	C	.836	.865	.822	.782	.799	.804	.825	.837	.803	.778	.857	.787	.795	.761	.877
	Ave.	.862	.830	.817	.817	.793	.813	.823	.847	.827	.798	.848	.811	.755	.771	.884
	s.e.	±.019	±.071	±.011	±.032	±.007	±.022	±.012	±.016	±.032	±.017	±.010	±.031	±.043	±.019	±.026
H popul[n.]	A	.793	.839	Separated tubes	.761	.582	.811	.807	.731	.826	.809	.842	.728	.771	.836	.964
	B	.844	.765		.895	.756	.875	.849	.548	.577	.755	.758	.835	.864	.815	.834
	C	.684	.869		.681	.818	.717	.781	1.260	1.108	.853	.915	.738	1.020	.718	.887
	Ave.	.762	.825		.776	.719	.801	.810	.846	.837	.805	.833	.767	.885	.791	.895
	s.e.	±.082	±.054		±.107	±.122	±.080	±.034	±.370	±.266	±.049	±.078	±.059	±.126	±.063	±.065

Generation 12: Average weight per fly not measured

[n.] Average weight per fly measured in mg.

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Table 7. Peppermint experiment - yield over 18 generations of selection expressed as biomass (x 10^{-3} g).

Treatment	Rep.	Generation 3	4	5	6	7	8	9	10	11	13	14	15	16	17	18
N control	A	132	204	204	139	150	219	253	162	250	268	276	244	141	256	206
	B	136	226	193	153	184	215	206	155	177	244	296	210	179	305	229
	C	92	276	225	165	146	218	220	159	235	271	296	221	167	340	248
	Ave.	120	236	207	153	160	218	226	159	221	261	289	225	162	300	228
	s.e.	± 14	± 21	± 9	± 8	± 12	± 1	± 14	± 2	± 22	± 9	± 7	± 10	± 11	± 24	± 12
K control	A	220	233	221	200	181	234	214	154	242	272	241	209	212	297	247
	B	218	240	271	193	197	211	224	163	208	266	279	186	201	295	234
	C	182	203	215	184	210	223	226	211	276	195	275	185	176	270	248
	Ave.	207	225	237	192	196	223	221	176	242	245	265	193	196	287	243
	s.e.	± 12	± 11	± 18	± 5	± 8	± 7	± 4	± 18	± 20	± 25	± 12	± 8	± 11	± 9	± 5
P control	A	198	182	225	148	154	222	228	167	203	276	239	154	98	226	195
	B	135	176	192	108	124	205	218	120	189	245	194	99	76	261	177
	C	138	243	217	169	131	217	221	190	233	260	246	159	163	233	190
	Ave.	157	201	211	142	136	215	222	159	209	260	226	137	112	240	187
	s.e.	± 21	± 21	± 10	± 18	± 9	± 5	± 3	± 21	± 13	± 9	± 16	± 19	± 26	± 11	± 5
M popul^{n}.	A	142	246	Separated tubes	169	220	258	284	239	218	246	234	169	163	203	198
	B	163	224		162	146	232	241	155	228	268	229	217	204	275	273
	C	147	238		139	139	210	228	242	317	285	258	200	253	208	210
	Ave.	152	236		157	168	233	251	212	254	266	241	195	207	229	227
	s.e.	± 8	± 5		± 9	± 26	± 14	± 17	± 29	± 31	± 11	± 9	± 14	± 26	± 23	± 24

Generation 12: Biomass not measured

Table 8. Test 1 - Number of emergent flies on a range of peppermint concentrations.

Treatment	Rep	Peppermint conc$^{n.}$ (% essence) 0	0.125	0.250	0.375	0.500
N control	A	156	167	183	77	67
	B	169	172	220	101	137
	C	160	275	248	212	191
	Ave.	161.7	204.7	217.0	130.0	131.7
	s.e.	± 3.8	± 35.2	± 18.8	± 41.6	± 35.9
M control	A	183	340	240	220	174
	B	210	284	253	183	207
	C	118	133	135	192	90
	Ave.	170.3	252.3	209.3	198.3	157.0
	s.e.	± 27.3	± 61.8	± 37.4	±11.1	± 34.8
P control	A	181	261	259	272	257
	B	108	185	114	120	69
	C	169	184	197	175	93
	Ave.	152.7	210.0	190.0	189.0	139.7
	s.e.	± 22.6	± 25.5	± 42.0	± 44.4	± 59.1
H popul$^{n.}$	A	226	297	253	266	228
	B	203	166	179	123	89
	C	228	268	282	257	276
	Ave.	219.0	243.7	238.0	215.3	197.7
	s.e.	± 8.0	± 39.7	± 30.7	± 46.2	± 56.1

Table 9. Number of emergent flies on a range of peppermint concentrations after 1 generation of lapsed selection on normal medium.

Treatment	Rep	Peppermint conc$^{n.}$ (% essence) 0	0.250	0.500
N control	A	326	341	189
	B	326	269	158
	C	305	193	108
	Ave.	319.0	267.7	151.7
	s.e.	± 7.0	± 42.7	± 23.6
M control	A	225	235	179
	B	210	169	113
	C	264	229	98
	Ave.	233.0	211.0	130.0
	s.e.	± 16.1	± 21.1	± 24.9
P control	A	296	266	131
	B	196	147	47
	C	298	208	112
	Ave.	263.3	207.0	96.7
	s.e.	± 33.7	± 34.4	± 25.4
H population	A	347	287	59
	B	288	236	184
	C	301	258	58
	Ave.	312.0	260.3	100.3
	s.e.	± 17.9	± 14.8	± 41.8

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Table 10. Test 4 on a range of peppermint concentrations after 15 generations of selection.

a) Number of emergent flies

Treatment	Rep	Peppermint conc$^{n.}$ (% essence) 0	0.125	0.250	0.375	0.500
N control	A	296	370	197	164	287
	B	304	236	233	197	71
	C	310	373	236	325	195
	Ave.	303	326	222	229	184
	s.e.	± 4.1	± 45.2	± 12.5	± 49.1	± 62.6
M control	A	313	264	291	486	216
	B	339	317	310	338	251
	C	280	254	355	251	228
	Ave.	311	278	319	358	232
	s.e.	± 17.1	± 19.5	± 19.0	± 68.6	± 10.3
P control	A	394	239	331	268	228
	B	353	334	219	258	169
	C	477	373	433	249	168
	Ave.	408	315	328	258	188
	s.e.	± 36.5	± 39.8	± 61.8	± 5.5	± 19.8
H popul$^{n.}$	A	379	304	293	234	166
	B	326	325	295	316	193
	C	339	290	208	261	258
	Ave.	348	306	265	270	206
	s.e.	± 15.9	± 10.2	± 28.7	± 24.1	± 27.3

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Table 10. Test 4 on a range of peppermint concentrations after 15 generations of selection.

b) Biomass (g)

Treatment	Rep	Peppermint conc$^{n.}$ (% essence) 0	0.125	0.250	0.375	0.500
N control	A	.180	.219	.133	.123	.216
	B	.208	.230	.206	.155	.063
	C	.216	.298	.188	.269	.182
	Ave.	.201	.249	.176	.182	.154
	s.e.	± .010	± .025	± .022	± .044	± .046
M control	A	.214	.209	.235	.386	.193
	B	.220	.262	.246	.254	.227
	C	.179	.209	.257	.207	.200
	Ave.	.204	.227	.246	.282	.207
	s.e.	± .013	± .018	± .006	± .054	± .010
P control	A	.282	.185	.249	.220	.204
	B	.182	.261	.165	.202	.138
	C	.306	.304	.294	.220	.156
	Ave.	.257	.250	.236	.214	.166
	s.e.	± .038	± .035	± .038	± .006	± .020
H population	A	.257	.211	.220	.219	.153
	B	.223	.283	.245	.276	.191
	C	.225	.237	.167	.222	.208
	Ave.	.235	.244	.211	.239	.184
	s.e.	± .011	± .021	± .023	± .019	± .016

Table 11. Test 5 on a range of peppermint concentrations after 15 generations of selection and 1 generation of lapsed selection on normal medium.

a) Number of emergent flies

Treatment	Rep	Peppermint conc$^{n.}$ (% essence) 0	0.125	0.250	0.375	0.500
N control	A	383	349	345	138	231
	B	314	275	187	271	253
	C	405	433	320	312	206
	Ave.	367.3	352.3	284.0	240.3	230.0
	s.e.	± 27	± 46	± 49	± 53	± 14
M control	A	357	347	282	+	230
	B	405	429	336	283	217
	C	217	+	228	+	255
	Ave.	326.3	388.0	282.0	283.0	234.0
	s.e.	± 56	± 41	± 31	± 0	± 11
P control	A	367	412	386	245	242
	B	269	398	249	183	88
	C	335	307	268	251	105
	Ave.	323.7	372.3	301.0	226.3	145.0
	s.e.	± 29	± 33	± 43	± 22	± 49
H population	A	392	356	334	311	143
	B	373	+	321	+	173
	C	345	359	300	356	187
	Ave.	370.0	357.5	318.3	333.5	167.7
	s.e.	± 14	± 2	± 10	± 23	± 13

Table 11. Test 5 on a range of peppermint concentrations after 15 generations of selection and 1 generation of lapsed selection on normal medium.

b) Biomass (g)

Treatment	Rep	Peppermint conc$^{n.}$ (% essence) 0	0.125	0.250	0.375	0.500
N control	A	.250	.282	.242	.124	.167
	B	.249	.235	.139	.224	.180
	C	.259	.326	.220	.235	.157
	Ave.	.253	.281	.200	.194	.168
	S.e.	±.003	± .026	± .031	± .035	± .007
M control	A	.232	.308	.199	+	.171
	B	.258	.330	.242	.223	.164
	C	.151	+	.156	+	.185
	Ave.	.214	.319	.199	.223	,173
	s.e.	± .032	± .011	± .025	± 0	± .066
P control	A	.261	.307	.272	.198	.170
	B	.187	.307	.198	.158	.078
	C	.233	.205	.228	.231	.100
	Ave.	.277	.273	.232	.196	.116
	s.e.	± .022	± .034	± .021	± .021	± .028
H population	A	.240	.266	.229	.256	.114
	B	.260	+	.238	+	.149
	C	.240	.244	.220	.278	.162
	Ave.	.247	.255	.229	.267	.142
	s.e.	± .007	± .011	± .052	± .011	± .014

Table 12. Test 3 - yield (number of emergent flies) on different heterogeneity levels.

Treatment	Rep	Heterogeneity level* 20N:0P	15N:5P	5N:15P	0N:20P	10N:10P	Within 10N:10P yield on N	P
N control	A	333	208	331	214	339	159	181
	B	333	237	331	218	374	197	177
	C	347	337	428	238	420	218	202
	Ave.	337.7	260.7	363.3	223.3	377.7	191.3	186.7
	s.e.	± 4.7	± 39.1	± 32.3	± 7.4	± 23.5	± 17.3	± 7.8
M control	A	316	315	409	246	465	254	211
	B	280	328	383	310	381	214	167
	C	220	244	325	167	428	258	170
	Ave.	272.0	295.7	372.3	241.0	424.7	242.0	182.7
	s.e.	± 28.0	± 26.1	± 24.8	± 41.4	± 24.3	± 14.0	± 14.2
P control	A	310	281	317	268	380	220	160
	B	278	249	273	210	374	215	159
	C	306	248	306	209	433	267	166
	Ave.	298.0	259.3	298.7	229.0	395.7	234.0	161.7
	s.e.	± 10.1	± 10.8	± 13.2	± 19.5	± 18.7	± 16.6	± 2.2
H popul$^{n.}$	A	294	327	490	250	448	271	177
	B	329	268	285	235	393	246	147
	C	250	243	252	181	411	213	198
	Ave.	291.0	279.3	342.3	222.0	417.3	243.3	174.0
	s.e.	± 22.9	± 24.9	± 74.4	± 21.0	± 16.2	± 16.8	± 14.8

Heterogeneity level* - Number of tubes/20 filled with normal(N) or peppermint (P) food.

APPENDIX 2

Table 13. Test 6 - yield on different heterogeneity levels after 16 generations of selection

a) Number of emergent flies

Treatment	Rep	Heterogeneity level 10N : 10N		15N : 5P		10N : 10P		5N : 15P		10P : 10P	
N control	A	235	208	349	93	130	110	133	216	+	+
	B	254	244	312	65	262	168	99	208	+	+
	C	229	205	327	61	99	63	109	203	36	55
	Ave.	239.3	219.0	329.3	73.0	163.7	113.7	113.7	209.0	36.0	55.0
	s.e.	7.5	12.5	10.7	10.1	50.0	30.4	10.1	3.8	0	0
	Sum aves.	458.3		402.3		277.3		322.7		91.0	
M control	A	137	175	378	65	208	230	126	221	142	130
	B	148	137	316	47	117	48	79	198	+	+
	C	200	133	358	106	223	190	154	273	142	112
	Ave.	161.7	148.3	350.7	72.7	182.7	156.0	119.7	230.7	142.0	121.0
	s.e.	19.4	13.4	13.3	17.5	33.1	55.2	21.9	22.2	0	9.0
	Sum aves.	310.0		420.0		338.7		350.3		263.0	
P control	A	192	197	304	112	150	138	111	193	164	115
	B	148	150	297	45	257	209	133	250	154	124
	C	188	257	245	186	137	208	117	262	+	+
	Ave.	176.0	201.3	282.0	114.3	181.3	185.0	120.3	235.0	159.0	119.5
	s.e.	14.0	31.0	18.6	40.7	38.0	23.5	6.6	21.3	5.0	4.5
	Sum aves.	377.3		396.3		366.3		355.3		278.5	
H popul^{n}	A	167	179	151	76	153	111	116	257	171	165
	B	243	153	323	109	115	112	135	195	223	183
	C	190	260	296	63	78	101	110	149	208	160
	Ave.	200.0	197.3	256.7	82.7	115.3	108.0	120.3	200.3	200.7	169.3
	s.e.	22.5	32.2	53.4	13.7	21.7	3.5	7.3	31.3	15.3	7.0
	Sum aves.	397.3		339.4		223.3		320.6		370.0	

b) Biomass (g)

Treatment	Rep	10N : 10N		15N : 5P		10N : 10P		5N : 15P		10P : 10P	
N control	A	.184	.150	.224	.078	.108	.090	.080	.162	+	+
	B	.188	.178	.241	.059	.178	.142	.074	.169	+	+
	C	.177	.150	.228	.058	.089	.051	.077	.173	.040	.051
	Ave.	.183	.159	.231	.065	.125	.094	.077	.168	.040	.051
	s.e.	.003	.009	.005	.007	.027	.026	.002	.003	0.0	0.0
	Sum aves.	.342		.296		.219		.244		.091	
M control	A	.124	.155	.248	.052	.147	.172	.074	.205	.115	.111
	B	.140	.135	.229	.045	.109	.047	.065	.191	+	+
	C	.155	.114	.240	.092	.202	.141	.095	.203	.118	.092
	Ave.	.140	.135	.239	.063	.153	.120	.078	.200	.116	.102
	s.e.	.009	.012	.006	.015	.027	.038	.009	.004	.001	.009
	Sum aves.	.274		.302		.273		.278		.218	
P control	A	.160	.172	.223	.077	.140	.106	.072	.142	.125	.090
	B	.128	.121	+	+	.200	.127	.086	.198	.116	.095
	C	.157	.138	.167	.130	.139	.176	.081	.207	+	+
	Ave.	.148	.160	.195	.104	.160	.136	.080	.182	.121	.093
	s.e.	.010	.020	.028	.026	.020	.021	.041	.020	.005	.025
	Sum aves.	.309		.299		.296		.262		.214	
H popul^{n}	A	.112	.176	.111	.054	.152	.108	.072	.253	.117	.132
	B	.229	.132	.229	.084	.107	.088	.082	.195	.173	.135
	C	.194	.200	.229	.039	.088	.075	.079	.151	.155	.144
	Ave.	.172	.169	.139	.059	.116	.090	.078	.196	.148	.137
	s.e.	.030	.020	.039	.013	.019	.009	.003	.030	.017	.004
	Sum aves.	.341		.249		.206		.274		.285	

Table 13c. Number of emergent flies - calculations for ratio diagrams.

Treatment	Rep	Ratio P/N 0.33	1.00	3.00
N control	A	0.266	0.846	1.624
	B	0.208	0.641	2.101
	C	0.187	0.677	1.862
M control	A	0.172	1.106	1.754
	B	0.149	0.410	2.506
	C	0.296	0.852	1.773
P control	A	0.368	0.920	1.738
	B	o.152	0.813	1.880
	C	0.759	1.518	2.239
H population	A	0.503	0.725	2.216
	B	0.337	0.974	1.444
	C	0.213	1.295	1.355

Table 13d. Biomass - calculations for ratio diagrams

Treatment	Rep	Ratio P/N 0.33	1.00	3.00
N control	A	0.348	0.833	2.025
	B	0.245	0.798	2.284
	C	0.254	0.573	2.247
M control	A	0.210	1.170	2.770
	B	0.197	0.431	2.939
	C	0.383	0.658	2.137
P control	A	0.345	0.757	1.972
	B	-	0.635	2.302
	C	0.778	1.266	2.556
H population	A	0.487	0.711	3.514
	B	0.367	0.822	2.256
	C	0.170	0.852	1.911

APPENDIX 2

Table 14. Test 7 - yield on different heterogeneity levels after 16 generations of selection and 1 generation of lapsed selection on normal medium.

a) Number of emergent flies

Treatment	Rep	Heterogeneity level 10N : 10N		15N : 5P		10N : 10P		5N : 15P		10P : 10P	
N control	A	194	263	369	157	223	194	142	219	109	117
	B	+	+	304	109	223	152	80	293	200	241
	C	137	152	244	160	259	219	138	132	174	91
	Ave.	165.5	207.5	305.7	142.0	235.0	188.3	120.0	214.7	161.0	149.7
	s.e.	28	55	36	17	12	20	20	47	27	46
	Sum aves.	373.0		447.7		423.3		334.7		310.7	
N control	A	145	132	290	143	238	219	144	260	159	188
	B	234	263	249	122	262	171	127	199	193	173
	C	168	223	207	79	259	198	48	284	116	210
	Ave.	182.3	212.7	248.7	114.7	253.0	196.0	106.3	247.7	156.0	190.3
	s.e.	27	32	24	19	8	14	30	25	22	11
	Sum aves.	395.0		363.3		449.0		354.0		346.3	
P control	A	243	278	301	129	189	198	152	212	261	197
	B	307	251	127	20	259	124	164	177	163	141
	C	111	246	380	91	200	62	145	124	96	138
	Ave.	220.3	258.3	269.3	80.0	216.0	128.0	153.7	171.0	173.3	158.7
	s.e.	58	10	75	32	22	39	6	26	48	19
	Sum aves.	478.7		349.3		344.0		324.7		332.0	
H popul^{n}.	A	180	199	294	84	94	109	130	118	116	214
	B	62	170	381	82	203	209	56	45	148	209
	C	197	276	235	120	141	222	129	193	211	154
	Ave.	146.3	215.0	270.0	95.3	146.0	180.0	105.0	118.7	138.3	192.3
	s.e.	42	32	72	12	32	36	25	43	28	19
	Sum aves.	361.3		365.3		326.0		223.7		317.3	

b) Biomass (g)

Treatment	Rep	10N : 10P		15N : 5P		10N : 10P		5N : 15P		10P : 10P	
N control	A	.148	.161	.285	.092	.164	.172	.090	.197	.093	.098
	B	+	+	.256	.089	.168	.139	.073	.240	.184	.203
	C	.121	.133	.172	.114	.171	.162	.090	.130	.153	.094
	Ave.	.135	.147	.238	.098	.168	.157	.081	.189	.143	.132
	s.e.	.018	.014	.034	.008	.002	.010	.006	.032	.027	.036
	Sum aves.	.282		.336		.325		.273		.275	
N control	A	.111	.115	.263	.092	.165	.163	.098	.220	.139	.149
	B	.185	.172	.226	.081	.195	.161	.100	.165	.160	.156
	C	.159	.165	.172	.066	.201	.163	.045	.227	.101	.178
	Ave.	.152	.151	.220	.080	.187	.162	.081	.204	.133	.161
	s.e.	.022	.018	.026	.008	.011	.001	.018	.020	.017	.009
	Sum aves.	.302		.300		.349		.285		.294	
P control	A	.162	.181	.238	.087	.139	.151	.097	.183	.192	.148
	B	.193	.167	.129	.018	.182	.105	.102	.162	.129	.124
	C	.097	.175	.266	.072	.194	.063	.099	.119	.079	.124
	Ave.	.151	.174	.211	.059	.172	.106	.099	.155	.133	.132
	s.e.	.028	.004	.042	.021	.017	.025	.001	.019	.032	.008
	Sum aves.	.325		.270		.278		.254		.265	
H popul^{n}.	A	.150	.162	.233	.064	.084	.112	.094	.112	.113	.169
	B	.040	.121	.274	.076	.173	.172	.043	.047	.176	.104
	C	.153	.174	.235	.095	.134	.142	.105	.167	.175	.138
	Ave.	.114	.152	.247	.078	.130	.142	.081	.109	.141	.137
	s.e.	.037	.016	.013	.009	.026	.017	.019	.033	.018	.019
	Sum aves.	.267		.326		.272		.189		.278	

Table 14c. *Number of emergent flies - calculations for ratio diagrams*

Treatment	Rep	Ratio P/N 0.33	1.00	3.00
N control	A	0.425	0.870	1.542
	B	0.359	0.682	3.663
	C	0.656	0.846	0.957
M control	A	0.493	0.920	1.806
	B	0.490	0.653	1.567
	C	0.382	0.764	5.917
P control	A	0.429	1.048	1.395
	B	0.157	0.479	1.079
	C	0.239	0.310	0.855
H population	A	0.286	1.160	0.908
	B	0.215	1.030	0.804
	C	0.511	1.574	1.496

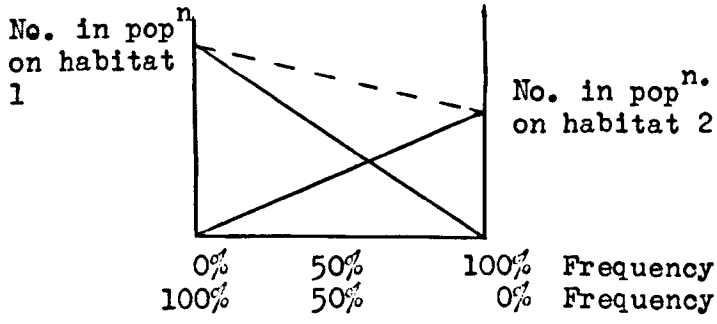
Table 14d. Biomass - calculations for ratio diagrams

Treatment	Rep	Ratio P/N 0.33	1.00	3.00
N control	A	0.323	1.049	2.189
	B	0.348	0.827	3.288
	C	0.663	0.947	1.444
M control	A	0.350	0.988	2.245
	B	0.358	0.826	1.650
	C	0.384	0.811	5.044
P control	A	0.366	1.086	1.887
	B	0.140	0.577	1.588
	C	0.271	0.325	1.202
H population	A	0.275	1.333	1.192
	B	0.277	0.994	1.093
	C	0.404	1.060	1.591

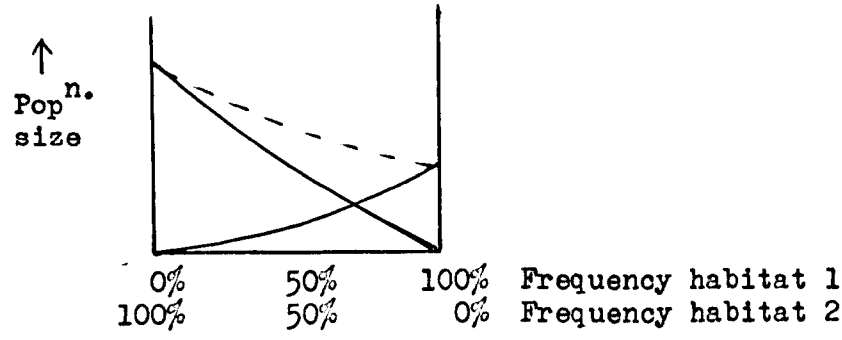
Analysis of tests 6 and 7

Replacement Series Graphs

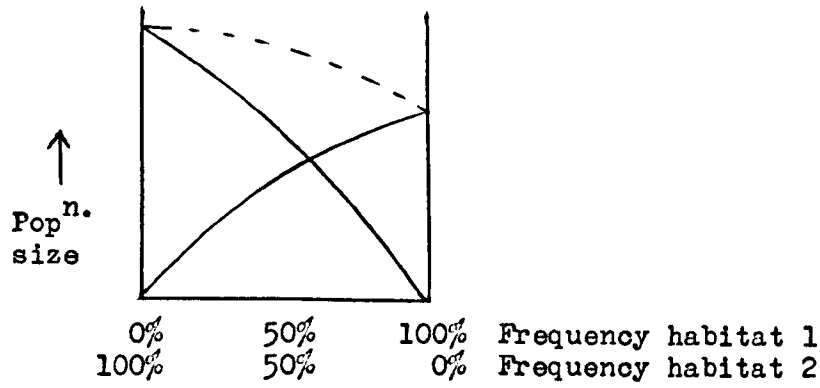
The analysis of De Wit (1960) was used to indicate the extent to which the different habitats in the heterogeneous environment contributed to the resultant population. The following diagrams show some theoretical graphs and their interpretation:



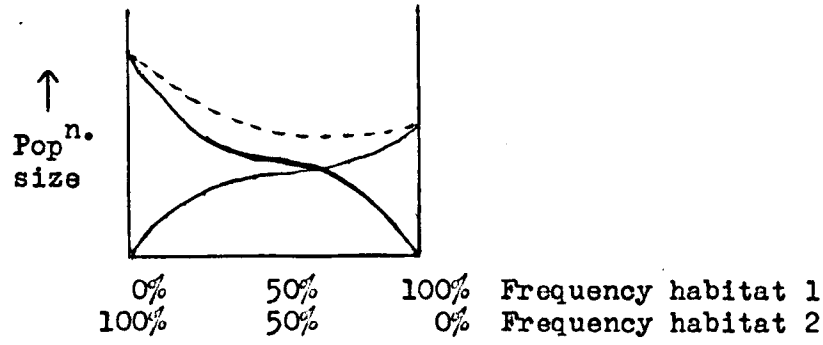
Population is adapted to habitat 1 (compared with habitat 2) but each habitat contributes proportionately equivalent numbers to the resultant population.



Population is adapted to habitat 1 (compared with habitat 2) but the numbers in both habitats are reduced in each other's presence. Therefore negative adaptation to heterogeneous situation.



Population adapted to habitat 1 (compared with habitat 2) but numbers increased in both habitats when both are present - positive adaptation to heterogeneous situation.

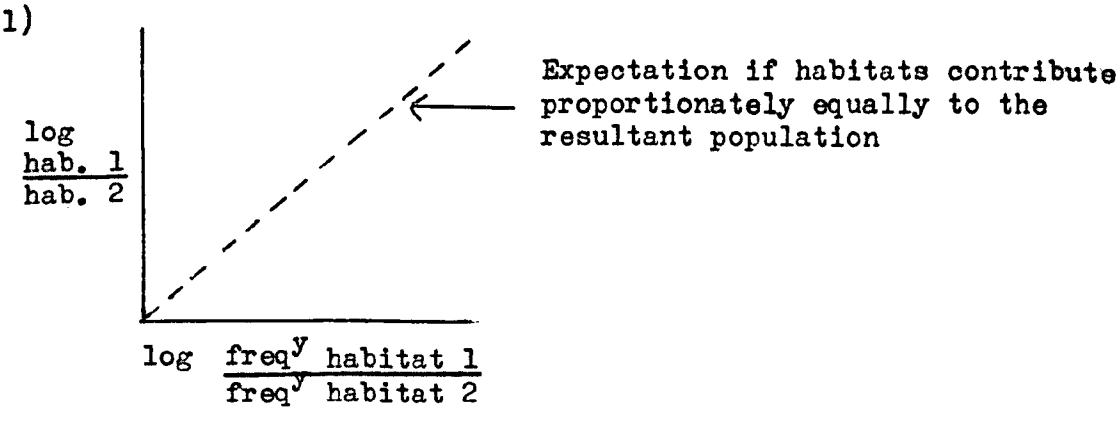


Population adapted to habitat 1 (compared with habitat 2) but overproduction of numbers on minority habitat in heterogeneous situation.

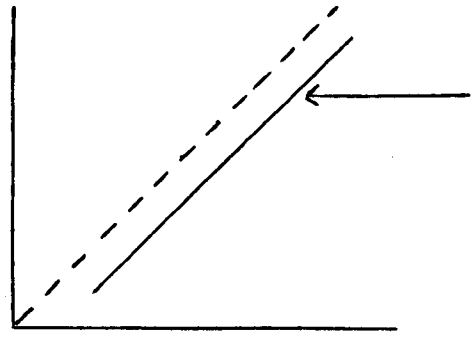
Ratio diagrams

Replacement Series Graphs may also be plotted as ratio diagrams on a log-log scale. The following diagrams show some theoretical graphs and their interpretation:

1)

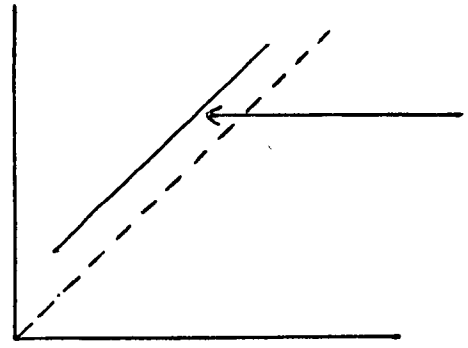


2)



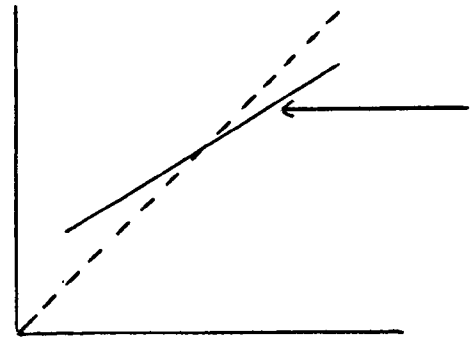
Habitat 2 contributes proportionately greater numbers to the resultant population than habitat 1 - therefore population adapted to habitat 2.

3)



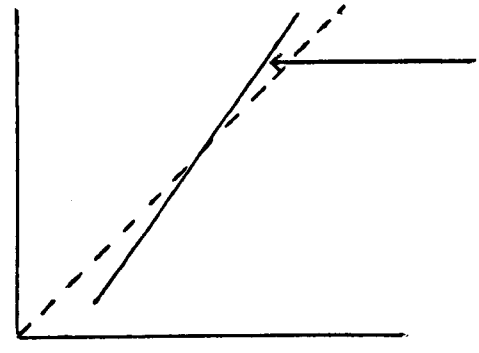
Population adapted to habitat 1

4)



Minority habitat contributes proportionately greater numbers to the resultant population than majority habitat.

5)



Majority habitat contributes proportionately greater numbers to the resultant population.

Graphs 1, 2 and 3, show uniformity of response of the members of the population to the environment. An individual might discriminate between the habitats and show preference for one of them, but not necessarily.

Graphs 4 and 5 indicate that an individual within the population distinguishes the 2 habitats and then (1) chooses that

habitat to which it is adapted or (2) chooses a habitat depending on its relative frequency within the environment. If (1) applies, then the population would consist of divergent strains, each adapted to one habitat. If (2) applies, then the population would consist of flies adapted to both habitats but able to discriminate between them.

APPENDIX 2

Table 15. Test 8 - egg production

Rep	treatment N control	M control	P control	H popul$^{n.}$	Within H popul$^{n.}$ HN	HP
1	265	281	260	225	182	43
2	335	292	167	295.	258	37
3	258	367	243	300	255	45
4	164	332	199	296	227	69
5	395	260	314	390	350	40
6	380	-	185	356	272	84
Ave.	299.5	306.4	228.0	310.3	265.7	52.5
s.e.	87.1	43.8	54.9	57.0	55.5	19.5