Some studies on genetical and environmental factors affecting the behaviour of *Drosophila melanogaster*

by

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CONTENTS

Introduction

Ethologists have observed that there are certain criteria which generally apply to instinctive behaviour patterns. For example the patterns are often released by extremely specific stimuli while the components of the patterns tend to be stereotyped in form. In order to account for these and other phenomena certain hypothetical mechanisms have been formulated. In the same way that comparative anatomists have been able to suggest some probable directions of evolutionary change in morphological characters so ethologists, by comparing behaviour patterns in related species, have been able to suggest the course of their phylogeny.

However before one can accept the validity of the hypotheses which have been evoked to explain ethological observations they must be shown to correspond with what is known about the way in which the nervous system acts. Similarly, an appreciation of the genetic basis of behaviour is a prerequisite to the proper understanding of its evolution. Thus there seem to be two fruitful extensions to "classical" ethology. One is the examination of the neurophysiological and hormonal basis of behaviour patterns that have already been studied from an ethological standpoint. The second is the investigation of the way in which the genotype controls these patterns.

The former approach is providing some interesting results. For example Lehrman (1961) has shown how the components of courtship, nest building and feeding the young and the sequence of

hormone production in the dove are interdependent. Huber (1962) by using techniques of ablation and stimulation has examined the neurophysiological basis of cricket song and has been able to suggest ways in which singing behaviour may have evolved.

I have been more interested in the second extension of ethology - the way in which the genotype controls behaviour. There are two obvious reasons why such investigations can be useful. First by carrying out selection experiments and by using certain quantitative techniques it is possible to estimate the amount of genetic variability accessible to selection and to evaluate possible directions and speeds of evolutionary change. Second, by modifying a behavioural character through artificial selection, it should be possible to examine the effect of such a change on other characters. Interactions of this type could tell one something about the organisation of behaviour within the animal.

The genotype must ultimately control behaviour in the same way that it controls morphological and physiological characters. The only difference that there is likely to be is that the number of intermediate steps between the genotype and its expression will tend to be greater for behavioural characters. However the genetics of behaviour has, until recently, been little studied except in man where genetic techniques have been used extensively to provide evidence in the nature versus nurture argument in traits such as intelligence and personality. Man, however, is not a convenient subject for genetical experiments and the results seem to show little more than the fact that such traits have some inherited basis. Recently there has been an increase of interest

in behaviour genetics in animals particularly on the part of American psychologists. Early reviews by Hall (1951), Fuller (1951) and Caspari (1958) have been followed by a book by Fuller and Thompson, 'Behaviour Genetics' (1960) and 'Roots of Behaviour' edited by Bliss (1962) contains further reviews by McClearn, Jakway, Bruell and others on aspects of the subject.

The previous lack of interest has probably been due to the following understandable reasons. Behavioural characters are less convenient than morphological ones in that while the latter are always available for measurement, certain types of behaviour, such as aggressive or sexual behaviour, may not always be easily elicited. Also it is often more difficult to break down behaviour patterns into meaningful units for the purposes of measurement. In the work described here the courtship of Drosophila melanogaster was chosen as a useful behaviour pattern as it to a large extent lacked these disadvantages. It is stereotyped in form and its components are easily recognised and recorded. Bastock and Manning (1955) have carried out an ethological study on the courtship behaviour of melanogaster. Further a vast amount of information exists on the genetics of this insect. These factors should make melanogaster an ideal subject for studies in behaviour genetics.

There is no need to provide a review of behaviour genetics but it is helpful to consider some of the different types of approach that have been employed particularly among workers using Drosophila species. These approaches can conveniently be grouped under the following headings:

a. Comparisons between species along with the examination of hybrids.

b. Comparisons of different genotypes within the same species such as geographical races and inbred lines and of crosses between them.

c. The effects of single gene mutations.

d. Selection for sexual isolation.

e. Selection for specific behavioural changes.

Comparisons between species

a. While there have been a very large number of comparative studies carried out in different groups of animals these have not often been followed by examination of hybrids between the species described. This is not a criticism of comparative ethology as hybridisation is seldom possible and a further limitation exists in that where hybrids can be obtained hybrid sterility almost always makes further genetic analysis impossible. One original comparative study, that of Lorenz (reviewed 1958) on the Anatidae, has recently been implemented by an examination of the courtship of hybrids between the Mallard, Black Duck and Florida Duck (Ramsay, 1961). In the hybrids most of the parental behaviour movements were inherited intact but appeared in novel sequences suggesting that the inheritance of the various movements is divorced from that of the co-ordination of the patterns. Hess (1962) quotes some unpublished data of von de Wall who has carried out similar experiments with hybrid ducks. The results are essentially the same as those of Ramsay. In addition, however, in some of the crosses motor patterns appeared which, while they were absent from both the parent species, were to be found in other related species. It is obvious from this

that the genetic potentialities for these patterns had not been lost but that their threshold had been raised so that they never normally appeared.

Other hybrid studies have been carried out, for example, with species of Callosamia (Haskins and Haskins, 1958), xiphophorin fish (Clark, Aronson and Gordon, 1954) and cardueline finches (Hinde, 1956), the characters under examination being cocoon spinning, sexual behaviour and, in the latter case, a range of characters including song and courtship behaviour. Mayr (1946), Spieth (1947, 1951) and Manning (1959a, 1959b) and others have compared the courtship behaviour of various species of Drosophila but of these only the latter has examined the behaviour of hybrids, in this case between the sibling species simulans and melanogaster. One general conclusion from these and other such experiments is that behavioural characters are almost always multifactorially controlled. If the behaviour is qualitatively similar in the parent species the hybrids are usually intermediate. One possible exception is described by Hormann-Heck (1957) who found that the mode of inheritance of certain characters concerned with courtship and aggression in two species of cricket, Gryllus campestris and G. bimaculatus, was consistent with a pattern of monofactorial transmission. As pointed out by Manning (1963b) this does not necessarily mean that the forms of the characters are themselves dependent on single genes. It is more likely that whether the character is to be expressed or not is controlled in a simple genetic manner.

Comparisons between genotypes

b. There are two advantages in using geographical races or inbred lines for genetical studies as opposed to experiments involving hybrids between species. If the behavioural traits in the parent species are qualitatively different they normally appear unmodified in the hybrid progeny or not at all. Such a result does not provide much information. On the other hand differences found within a species are usually quantitative and it is possible to use normal genetical techniques to learn something about continuously varying characters. Second, the degree of isolation is not generally great enough to interfere with a complete genetical analysis. Inbred lines have been used quite extensively by behaviour geneticists. For example McClearn and Rodgers (1959, 1961) have started examining the genetic basis of alcohol preference in mice, and Jakway (1959) and Goy and Jakway (1959) have studied the inheritance of patterns of mating behaviour in guinea pigs, both sets of workers using inbred lines. One suggestion from the latter work is that different aspects of mating behaviour are controlled by separate sets of alleles, a conclusion which is supported by the observations on hybrids. The use of inbred lines and of crosses between them allows one to make estimates of the dominance and heritability of characters along with the number of genes that are controlling them. Broadhurst and Jinks (1961) using the methods of biometric genetics have reanalysed some published data on, for example, activity in rats and mice and the work of Goy and Goy and Jakway mentioned above.

Apart from the work on inbred lines little has been done

comparing other populations such as geographical races, although there is sufficient evidence from sexual isolation studies, particularly in Drosophila species, that such research would be rewarding. Kessler (1962) reports large differences in the courtship behaviour of geographical races of D. paulistorum but has not extended the study to crosses between them. Spiess and Langer (1961) have shown that mating success is different in strains of D. persimilis which are polymorphic for inversions on chromosome III.

Single gene effects

c. A potentially useful technique is to examine the effects on behaviour of single mutant genes. One of the advantages of this class of experiment is that the exact nature of the genetic differences are known which is very seldom the case with more complex genetic systems. Once again workers such as Merrell (1949a, 1949b) and Rendel (1951) interested in sexual isolation have examined the results of substituting genes on sexual success in species of Drosophila. A more complete behavioural analysis has been carried out by Bastock (1956) who examined the effects on mating behaviour of the mutant gene 'yellow' in D. melanogaster. She demonstrated that the reduced sexual success of males carrying this gene was due to a reduction in courtship intensity. She further showed that females from the yellow stocks were more receptive than the wild type females and that this was not due to the direct effect of the mutant gene and suggests that there had been selection for increased receptivity to compensate for the

deficient courtship to which the females had been exposed. Petit (1958) has examined the effects of the mutant genes 'bar' and 'white', also on courtship behaviour, and found that the sexual success of these mutants in competition with the wild type males was in some way dependent on the relative proportions of the genotypes.

Single gene effects have not been much studied in animals other than Drosophila but Keeler and King (1942) reported a correlation between coat colour genes and docility in the rat. They did not, however, ensure genetic homogeneity in their strains and these results have not been confirmed.

Selection for sexual isolation

d. Selection for sexual isolation has been attrmpted with partial success several times in Drosophila. Knight et al. (1956), by eliminating the wild type hybrids, increased the sexual isolation between two stocks of D. melanogaster homozygous for the mutant genes 'ebony' and 'vestigal' respectively. Pearce (per. comm.) has repeate this experiment and states that differences in the micro-habitat preferences of the mutants was influencing the results as much as differences in sexual behaviour. Selection for increased sexual isolation has been most convincingly demonstrated by Koopman (1950) who, by removing hybrids from a mixed population of D. pseudoobscura and D. persimilis, increased the degree of isolation between the two species. He also cursorily examined the behaviour of the flies to show that this was due mostly to increased discrimination on the part of the persimilis males. These experiments provide models to show how behavioural changes may help to maintain and

increase sexual isolation between incipient species either by affecting mating preferences or habitat choice.

Selection for specific behavioural changes

e. One of the earliest and best known examples of selection for a behavioural character is that of Tryon (1940) who selected lines of 'maze dull' and 'maze bright' rats. This experiment illustrates the usefulness of such selections in that these lines have subsequently been extensively used by other workers to investigate learning processes. For example Krechevsky (1933) showed that Tryon's brights tended to use spatial clues when running a maze while the dulls used visual clues. Searle (1949) also compared these lines with regard to a large number of variables and found that there were differences between the lines in measures of emotionality and motivational state.

Manning (1961) and Hirsch and Boudreau (1958) have used selection to change mating speed and phototaxis, respectively, in D. melanogaster and Woodrush (1960) has successfully selected for high and low sex drive in cockerels. All these experiments suggest that genetic variability, which is accessible to selection, exists for behavioural characters. The existence of this variability means that natural selection pressures are maintaining these characters at advantageous levels and the selection experiments indicate some of the many possible directions of evolutionary change.

I was introduced to the work described in this thesis through an observation made by Dr. F.W. Robertson who noted that there appeared to be differences in the mating speeds of lines of flies

that had been selected for changed body size. The differences in courtship behaviour that I found to be associated with changed body size led me to investigate further the way in which modifying the genotype could affect behaviour. I used some of the approaches that have been described previously such as selection experiments and the comparison of different genotypes and in order to assess the relative importance of genotype and environment in determining behaviour I attempted to apply some of the techniques of quantitative genetics. I have also looked at the effects of certain environmental factors on the flies' behaviour. There were two reasons for this. First, considerable variability, which is environmental in origin, is found in the measures employed and I wished to see whether this could be reduced. Second, although a theoretical separation can be made between the genotypic and environmental components of variance for a character, it is obvious that neither aspect can be ignored as both contribute to the overt behaviour of an animal.

My initial interest was in the genetic basis of courtship behaviour and some reasons for using such behaviour as the material for these investigations have already been given. However, early in these studies it was noted that there appeared to be differences in activity levels between some of the lines under examination and I therefore decided to investigate activity further. It is not difficult to imagine ways in which changes in activity could be of adaptive significance. Activity levels would influence dispersion and the chances of finding mates and suitable food sources. There may also be some connection between courtship and activity. Manning

(1959b) in comparing D. simulans and D. melanogaster states that the latter species is more vigorous in both its sexual behaviour and its activity. He also records the reverse situation in lines of melanogaster selected for fast and slow mating speed, the fast mating lines being less active than the slow mating lines. It might be possible, by changing the activity levels, and then examining courtship, to clarify the relationship between these two aspects of behaviour.

One would also like to obtain a clearer idea of what activity involves as it has been the object of some genetical research. Rundquist (1933) selected lines of rats for high and low levels of running activity and Thompson (1953) demonstrated that inbred strains of mice differed in their levels of exploratory activity as measured in an 'open field'. Measures such as exploration and running must be influenced by many factors and by examining the effects on activity of changing the environment and the genotype one might obtain some information on how activity is controlled. Melanogaster is possibly a better experimental animal than rats or mice for such an investigation as its behavioural responses are likely to be more simple although one is presented with the problem of extrapolating conclusions from invertebrates to vertebrates.

As a working definition of activity I would suggest 'the observable activity exhibited by an animal when not specifically activated by external stimuli'. It is impossible to eliminate all environmental stimuli but at least they can be minimised, although the experimental situation, being a novel one, could activate both fear and exploration.

Note on Appendices

The raw data from which the tables and figures are derived along with the results of the statistical analyses that were carried out are included in appendices. Each table and figure refers, when relevant, to the appropriate appendix.

CHAPTER I

Materials and Methods

1. Introduction

Except for a few special cases the methods for culturing and handling flies were standardised for all the experiments. I also standardised certain techniques used in quantifying the two aspects of behaviour with which this thesis is mainly concerned, namely courtship and activity. These methods are therefore dealt with in this section and any divergences from the usual procedures, along with special techniques, are described in the text as they arise.

2. Stocks

The base stock that was used in almost all the experiments was one obtained from Dr. F.W. Robertson of the Institute of Animal Genetics in Edinburgh and named by him "Pacific". The sub-culture that I maintained was kept in two or more half pint stock bottles and was continued each generation from at least fifty pairs of flies. Occasionally flies from the parent stock, which was kept in population cages, were added to my stocks in order to counteract any possible inbreeding effect. In theory the flies that would be least variable and therefore most suitable for behavioural analysis are the F1 progeny of crosses between inbred lines. Such flies, however, would not provide a good basis for selection experiments and also I shall present evidence later to show that in practice they may exhibit greater variance in certain measures than do out-

bred flies. I therefore used the Pacific stock which was kept deliberately heterozygous.

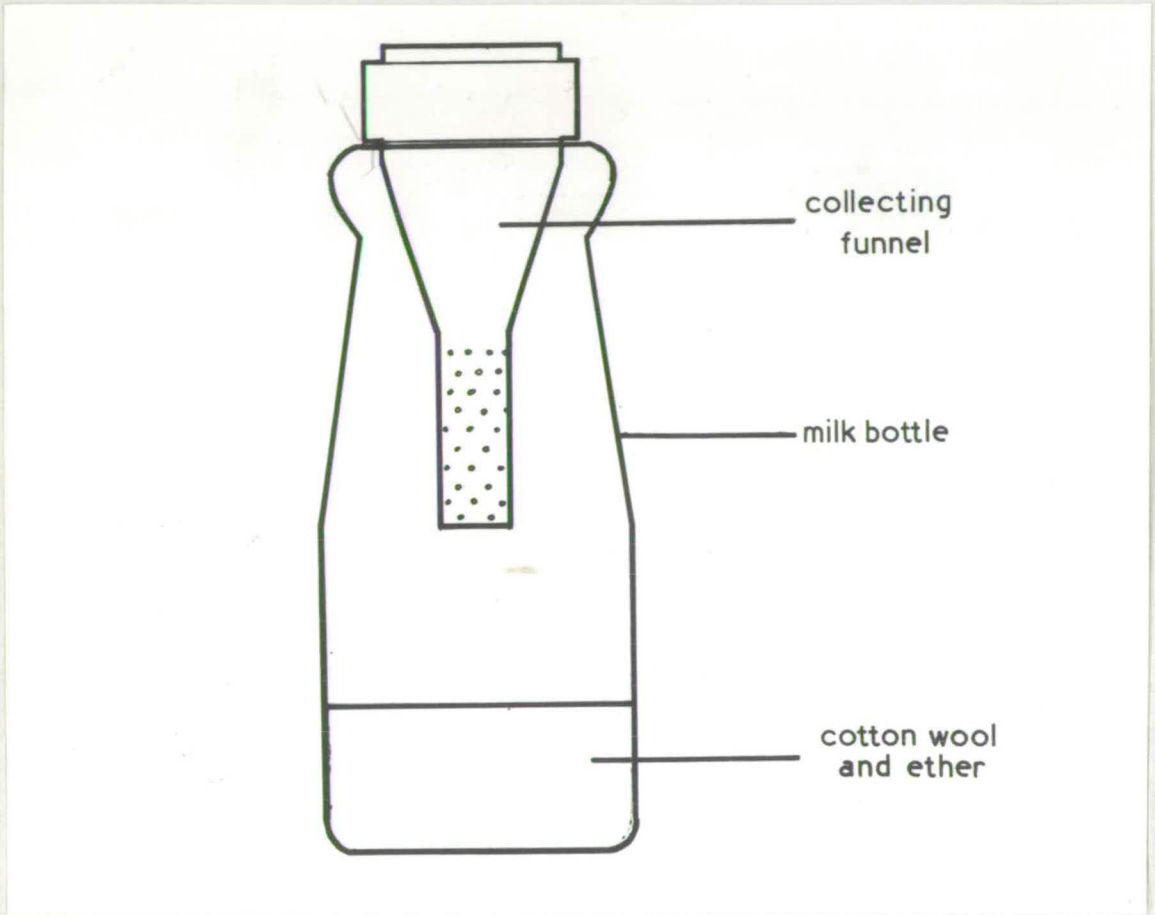
3. Methods of Culturing, Collecting and Handling the Flies

The standard agar, molasses and yeast medium was used to raise the larvae and to feed the adults. This I obtained from the Institute of Animal Genetics either in half pint milk bottles or in 3 x 1 inch vials. The former contained about one inch of medium in the bottom and these were used exclusively for rearing the larvae. When flies were required for experiments ten pairs of mature flies, from three to five days old, were introduced into one of the stock bottles and allowed to mate and lay eggs for 48 hours. This ensured that approximately 150 to 200 flies would emerge during the first two days of hatching which was normally sufficient for most experiments. The larvae were not overcrowded under such conditions and the flies were of a reasonably uniform size and with a common environmental background.

The flies were kept in the 3 x 1 inch vials which contained approximately 0.5 of an inch of medium. To this I usually added a few drops of a suspension of live yeast in water to provide additional food for the flies. The flies were knocked over on to fresh food each day until they were used in an experiment.

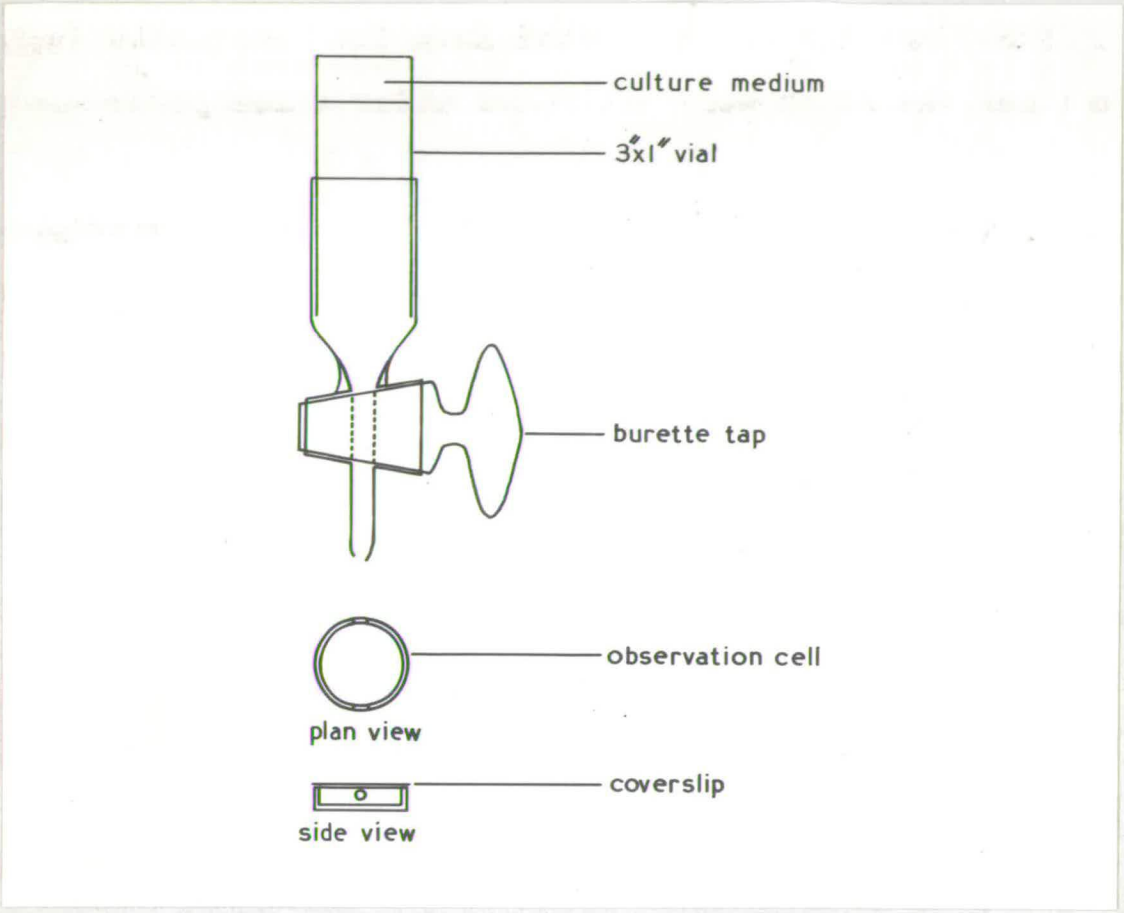
The flies were reared, matured and experimented on in a constant temperature room which was kept at 26 ± 1°C. A time switch automatically turned on two 100 watt bulbs at 9 a.m. and off at 11.30 p.m. every day. Under these conditions the generation time, from egg laying to the hatching of the imagos, was nine days.

Fig. 1



The apparatus used for collecting and etherising flies.

Fig. 2



The modified burette tap for introducing individual flies into the various types of apparatus used. Also illustrated is a perspex cell used to observe courtship behaviour.

The standard form of etheriser, which is illustrated in Fig. 1, was used to anaesthetise the flies on collection from the stock bottles. The cotton wool in the bottom of the bottle was soaked in ether and the flies knocked into the removable funnel. Once etherised the sexes were separated under a low power microscope.

I collected flies that were going to be used in experiments three times daily from the stock bottles at approximately 09.30 hrs., 16.30 hrs. and at 23.30 hrs. The sexes were separated once the flies had been etherised and were put in vials, from 10 to 50 per vial depending on the requirements of the experiment. This ensured that the flies used were all virgins as they do not normally mate during the first twelve hours after hatching. As an additional precaution, in for example selection experiments where the use of virgin females was particularly important, I kept the vials in which females had been stored to see whether any larvae appeared. If they did I discarded the females that had been kept in the vial.

Before they were used in any experiment the flies were kept until they were three or four days old. This is especially important in females as at that age they are at the peak of sexual receptivity (Manning, 1959a). Also, all the behavioural measures were carried out between 0900 hrs. and 1230 hrs.

Individual flies were handled by means of modified burette taps, one of which is illustrated in Fig. 2. The flies were tapped down to the bottom of the vial, the cotton wool plug removed, and the burette tap placed over the vial. When disturbed the flies

tend to run upwards and towards light. Thus the tap could be opened to allow one fly to pass through and then find the aperture at the end of the tube which would be held against a corresponding hole in the apparatus. Courtship behaviour often starts within a few seconds of the flies being so handled indicating that they are not unduly disturbed.

4. Courtship Behaviour and its Measurement

i. Description of Courtship

The courtship behaviour of D. melanogaster has been previously described by several workers, the most notable of whom have been Sturtevant (1915), Speith (1952) and Bastock and Manning (1955). The methods which I used to examine and analyse this behaviour are described in the next section and the behaviour pattern itself is as follows. When the male first meets a female he taps her on the body with his fore tarsi. Presumably this enables the male to recognise a potential mate (Speith, 1952) and once performed the movement is not normally repeated during the course of the courtship except following an unusually long break. I therefore ignored this movement in the records that I made. The next element in courtship has been termed orientation. This can be recognised by the male standing with his head pointing towards the female a few millimetres from her. If she is stationary, the male usually orientates towards the female's head, but more often the female is walking and then the male follows closely behind her. These two aspects were not separated by Bastock and Manning (1955) as which occurs depends only on the behaviour of the female and not of the male. Super-

imposed upon orientation is vibration. In this the male extends one wing horizontally and at right angles to his body and vibrates it in the vertical plane. The wing used seems usually to be the one nearest to the female's head but when following the male may alternate wings. If the female stops the male often circles to her head continuing to vibrate. Thirdly the male will extend his proboscis and 'lick' the female's genital plates. This is termed licking and is normally accompanied by the male curving his abdomen under his body and mounting or attempting to mount the female. These three courtship elements, orientation, vibration and licking are distinct and easily recognisable for the purposes of recording.

Bastock and Manning (1955) present evidence which suggests that the sequence of the elements represents increasing thresholds of sexual excitation in the male. Because of this evidence the term 'courtship intensity' can be used to compare courtships. A high intensity courtship is one which contains a high proportion of licking and vibration, these two elements being positively correlated with one another and negatively correlated with orientation. Conversely a low intensity courtship is one exhibiting a high proportion of orientation.

Each courtship element is not triggered off by a specific stimulus from the female as is found, for example, in the Three-spined Stickleback (Tinbergen, 1951). Possibly the only positive action on the part of the female is the spreading of the genital plates which allows the male to copulate. This, however, is not an acceptance posture such as is found in D. fumipennis (Spieth, 1947) in that it does not signal to the male the female's

preparedness to mate and the male will attempt to mount whether the vaginal plates are spread or not. The female may nevertheless exhibit several repelling movements which tend to inhibit male courtship. These consist of kicking the male, running or jumping away from him, flicking the wings and extrusion of the ovipositor. The latter must have a particularly strong inhibitory effect on the male as it usually interrupts courtship for a period. It is most often shown by fertilised females which will not mate for several days after insemination and the movement is therefore highly adaptive. Bastock and Manning (1955) suggest that the females are at the same time providing both inhibitory and excitatory stimuli and it is the interaction of these variables along with internal factors which cause the fluctuations in thresholds found during courtship.

ii. Single pair matings

Bastock and Manning (1955) have described a method of recording single pair courtships and this was the method that I employed. The courtships were observed in circular perspex cells one inch in diameter and one quarter of an inch deep and covered by glass coverslips. Two holes, 180° apart, were bored in the wall of the cell. One was plugged with absorbent cotton wool, which was saturated to keep the humidity in the cell high, and flies were introduced through the other in the manner already described. Small plastic pegs were used to plug this aperture. A low power binocular microscope whose field covered the entire cell was used to observe courtship. A metronome was set at eighty ticks per minute and

the courtship movement observed was recorded, by means of a code, on a typewriter every second beat of the metronome. Every courtship was recorded for at least 2.5 minutes, that is 100 units of courtship, unless copulation terminated courtship earlier. Any courtship with less than 20 units was discarded as providing too small a sample but many of the courtships covered the desired 2.5 minutes.

I analysed each courtship by calculating the percentages of each of the courtship elements, orientation, vibration and licking. This differed from the method of Bastock and Manning in that they used bout lengths of the elements. My reasons for so doing were as follows.

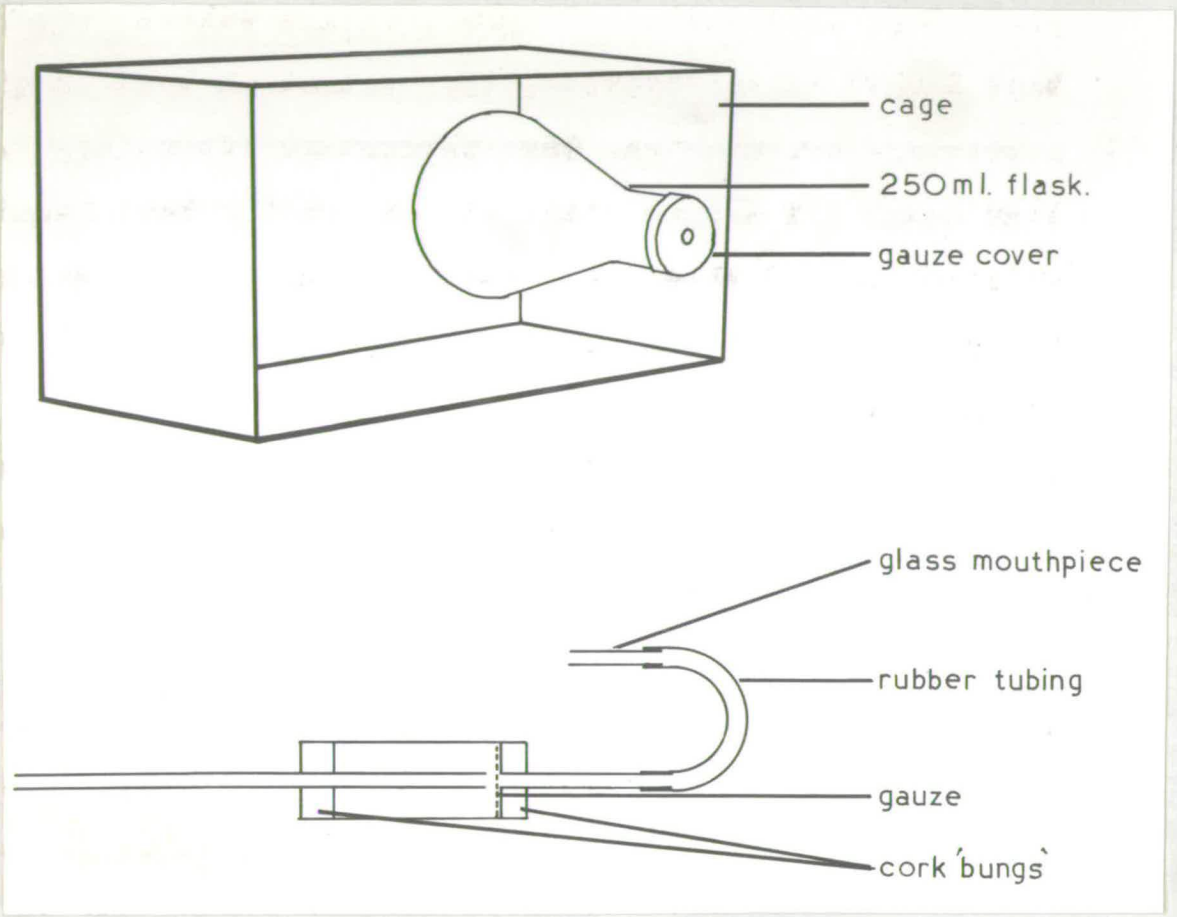
a. Percentages of the elements are more easily calculated than are their bout lengths.

b. Bastock and Manning used simulans females which would not copulate with melanogaster males. I used mature melanogaster females which sometimes did copulate within the test period and for short records percentages give less variable results than do bout lengths.

c. Bastock and Manning were using normal wild type males with reasonably high intensity courtship while certain of the flies from selected lines that I used exhibited very low intensity courtship. Using bout length in these cases often distorts the results. For example consider two hypothetical courtships having the following patterns:

(i) 49 units orientation, 2 units vibration, 49 units orientation.

Fig. 3



The apparatus used for mass matings (only one 250 ml. flask is shown for clarity) along with a 'pooter' used to remove pairs which had copulated.

(ii) 32 units orientation, 2 units vibration, 32 units orientation, 2 units vibration, 32 units orientation.

By the two methods we get:

(i) percentage orientation, 98; percentage vibration, 2. bout length of orientation, 49; vibration bout length, 2.

(ii) percentage orientation, 96; percentage vibration, 4. bout length of orientation, 32; vibration bout length, 2.

From a behavioural aspect these two courtships are very similar but if bout length of orientation is used to compare them the difference is greatly exaggerated.

d. Cane (1961), re-examining Bastock and Manning's data, gives statistical reasons as to why percentage orientation is a better measure of courtship intensity than is bout length.

iii. Mass Mating Method

The mass mating method has been described by Manning (1961). It is a measure of the mating speed of a population of flies and can be used as a quick and easy means of comparing two samples of flies to show whether there is any difference in sexual behaviour between them which might repay closer examination. Mass matings do not tell one whether any differences revealed by this method are due to changes in the behaviour of males or females.

The method that I used differs but slightly from that of Manning and the apparatus is illustrated in Fig. 3. A wire cage covered with white paper which transmitted diffuse light was made sufficiently large to accommodate two 250 ml. conical flasks. Lights were shone from behind and above the apparatus so that the

flasks were reasonably uniformly illuminated. This was thought necessary as flies tend to move towards the light source. Thus apparent differences in mating speed could be due to differences in this response. Flies which are strongly positively phototactic will tend to gather nearest the light source and will thus find mates more easily than flies less attracted to the light which will remain spread out in the flask. Manning (1961) found this happening during the early stages of selection for mating speed. The two flasks were held horizontally in position inside the cage by means of two retort stands. The mouths of the flasks were covered with gauze with a hole at the centre to admit the tube of a pooter (also shown in Fig. 3). Fifty male and fifty female flies were knocked into a flask which was then clamped into position. Pairs of flies were removed from the flask with a pooter as they copulated and the numbers noted at two minute intervals. Accommodation for two flasks meant that two samples could be measured simultaneously.

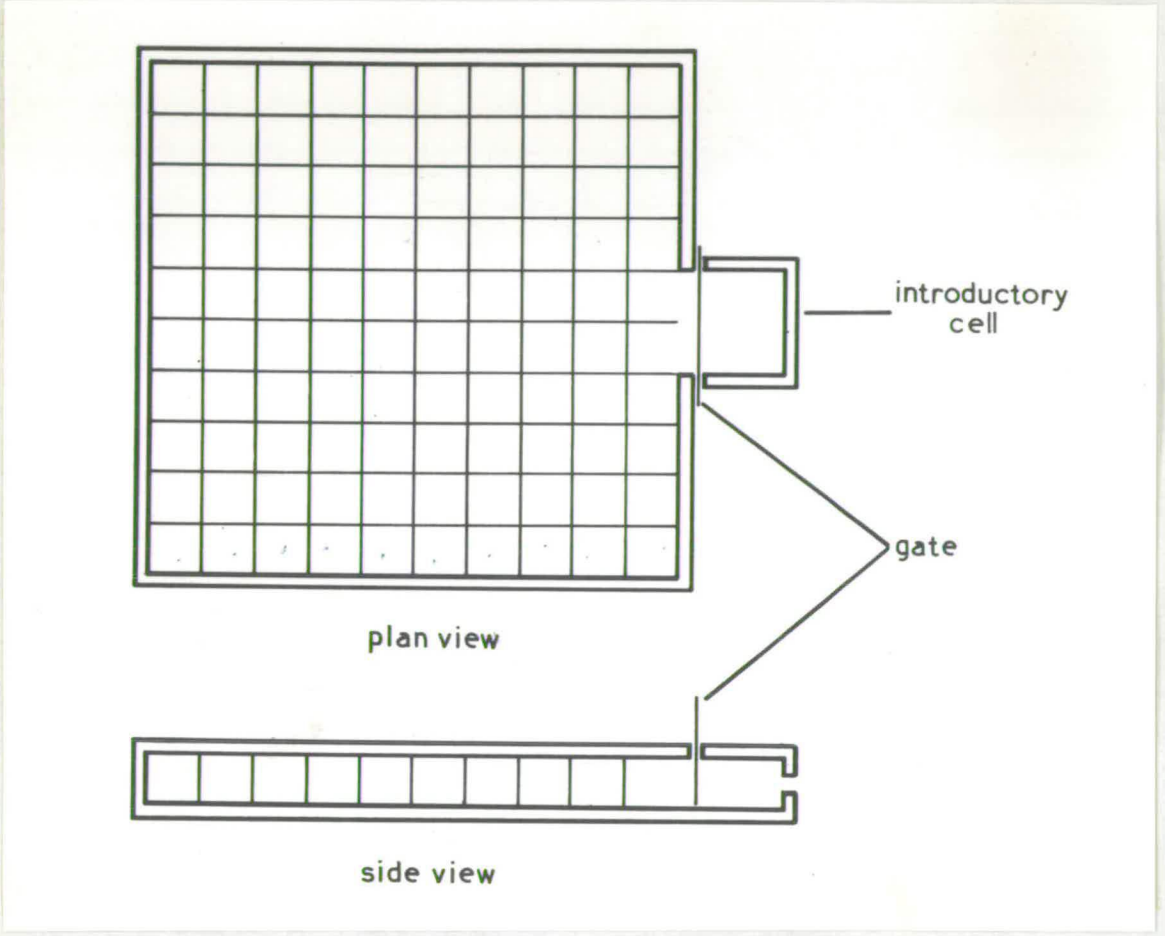
The experiment was usually terminated after 18 or 20 minutes as, in certain cases, almost all the flies had copulated by this time. The results were expressed as the cumulative number of copulations against time.

5. Activity and its Measurement

i. Introduction

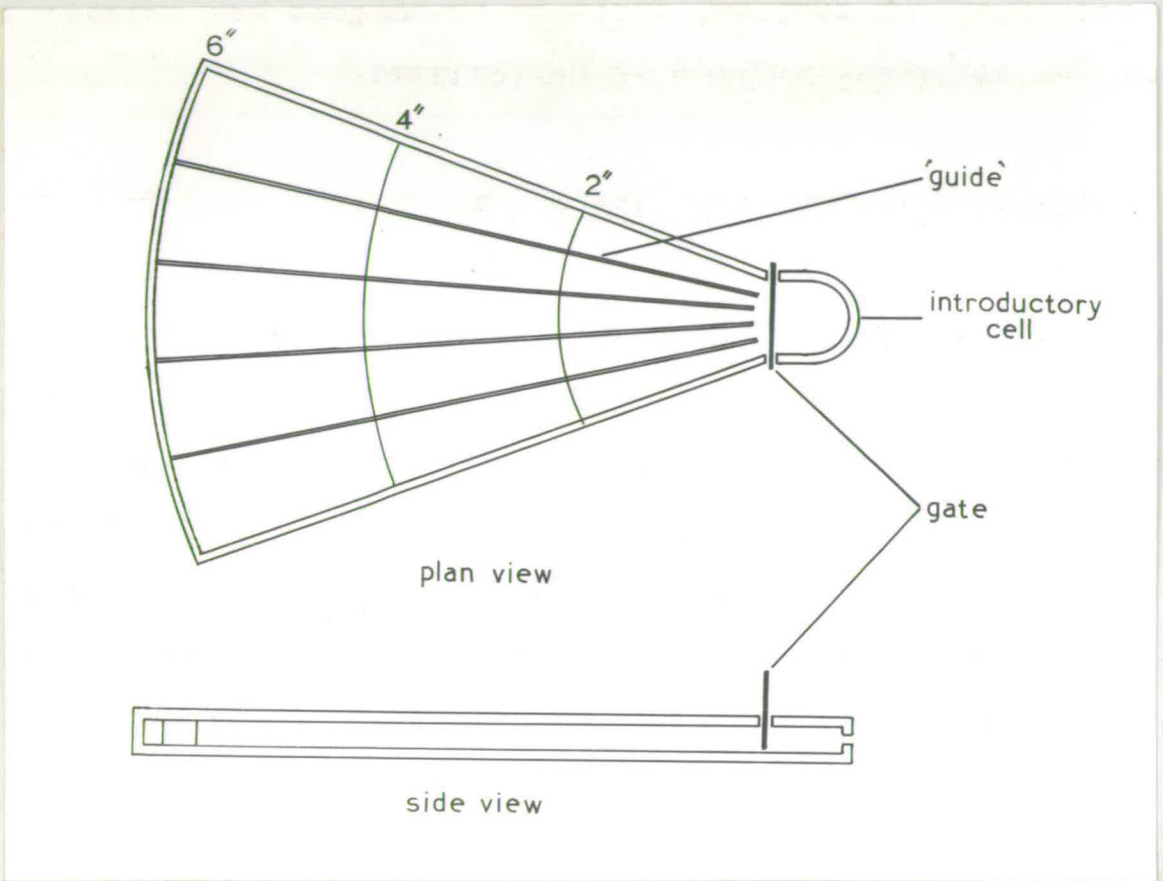
Probably the best way of measuring activity would be to examine the activity of individual flies over a long period of time, even several days, by means of an activity wheel and electronic

Fig. 4



The perspex arena in which activity was measured.

Fig. 5



The sector apparatus for measuring activity.

counter as employed by Roberts (1960) to measure activity in Cockroaches. Drosophila, however, is rather too small to use in this and other similar types of apparatus although technically it might be possible, and simpler, speedier techniques are necessary. I used the two following methods of measurement.

ii. Activity as measured in the arena

The apparatus used in this test is illustrated in Fig. 4. It consists of a perspex arena 10 cm. x 10 cm. x 1 cm. marked off in centimetre squares. A small cell, separated from the arena by a gate was attached to one wall and it was through this that flies were admitted into the arena. A fly was introduced into the cell and allowed to recover from the effects of manipulation for about 30 seconds. The gate was then lifted and the fly allowed to enter the arena. The number of squares that it entered, whether on the walls, floor or roof, during a one minute period was counted. In this confined space the flies did not normally fly and the measure is purely one of ambulatory activity. A single light directly above the arena was arranged to give a uniform light over the apparatus.

iii. Sector method of measuring activity

A second method, based on a slightly different principle, was also used to measure activity. The apparatus, which is made from perspex, is illustrated in Fig. 5. This represents the sector of a circle and the measurement made is of the rate of dispersal of flies from the apex of the sector. Five flies at a time were

introduced into the cell at the apex and, after a period of 30 seconds, the gate was removed allowing the flies into the sector. The sector was marked off at 2, 4 and 6 inch radii and the number of flies in each of these two inch segments and also those that had not left the cell were counted after 10 seconds. Three 'guides' ensured that the flies would run reasonably straight away from the apex and they also reduced the interference between the flies. Observation of the flies in the apparatus confirmed that the guides were successful in at least the former function. As in the previous apparatus a light was shone from directly above, uniformly illuminating the sector.

There are two main differences between this apparatus and the arena. First there is a difference in the length of time that the flies are tested. Second, in the sector interaction between the flies is not eliminated. The second difference would seem to be an important one and this aspect will be discussed later as will the interpretation of results obtained from these two measures.

CHAPTER II

The Normal Sources of Variance

1. Introduction

For considerations of experimental design the variance of behavioural characters can be split into three categories: within day variance, between day variance and between generation variance. The factors which cause the variance are not necessarily different in each case although some of them will be. For example, the between generation variance could be due, in part, to changes in the genotype while, as will be shown later, a diurnal rhythm in a behavioural character can increase the within day variance. We know that the between generation variance can be extremely large and an example is the fluctuation found by Manning (1961) in mating speed. This is not unique to behavioural characters for Hunter (1959) reports equally large fluctuations during the course of a selection experiment for length of larval period. These fluctuations are not however a serious hindrance as long as experimental results over a period of several generations are expressed as deviations from a suitable control and not as absolute values.

Between day variance can also be eliminated or allowed for. In the case of activity measures, a sufficiently large number can be carried out in one morning to show whether any differences exist between samples. Single pair matings, however, are more time consuming and often have to be spread over two or three mornings. Most people who have worked on courtship behaviour in *Drosophila* would agree that there are 'good' and 'bad' days for courtship.

Again this can be counteracted by examining control and experimental animals on each of the days.

Finally within day variance can be allowed for by the same means, that is, by testing control and experimental animals alternately or, in the case of mass matings, at the same time.

It is possible, by the means outlined above, to allow for the variance between days and generations and within days and thus demonstrate differences between samples which might otherwise be obscured. The origin of all the variation encountered in a population is, however, important. It might be due to differences in the previous environments of the animals. In this way for example differences in behaviour due to learning could affect the animals' responses to the experimental situation although this is probably not a large source of variance in Drosophila where previous influences are likely to be developmental in origin. It is particularly interesting to be able to partition the total variance into genetic and environmental components as the former in part provides a measure of the potentialities for selection and the latter the possibilities for individual adaptation.

Using techniques borrowed from quantitative genetics it is theoretically possible to divide the variance of a character into several components. The total phenotypic variance, VP, can be split into two main parts, genotypic variance, VG, and environmental variance, VE. The latter can be further subdivided into VEg, general environmental variance, and VEs, special environmental variance. The significance of this division is explained in the next section, that on repeatability. Although VG also consists

of two components, additive and non-additive, I did not attempt this separation.

2. Repeatability

i. Introduction

The measure of repeatability separates VEs from the phenotypic variance and is expressed as a ratio whose formula is $\frac{VG + VEg}{VP}$. VEs is, in behavioural measures, that portion of the variance due to the animals' changed responses to fluctuations in the environment, both external and internal to the animals, during the course of the experiment. VEg on the other hand is due to more general and prolonged differences in the environment during the time previous to the experiment and particularly during development. This ratio can be calculated for characters which are repeated either temporally or spatially in the same individual. An example of a spatially repeated character for which this has been calculated is bristle number on the ventral surface of the abdominal segments in D. melanogaster (Reeve and Robertson, 1954). Most physiological and possibly all behavioural characters come into the category of temporally repeated characters.

Repeatability can provide three useful pieces of information. First, an estimate of VEs which is the within day variance or, more accurately, the variance to be expected during the period of testing. Second, it reflects the accuracy of the method of testing in that, if repeatability is high the experimental error must be small. Finally it tests the validity of the measure used. For example, activity, as measured in the arena, is probably the

outcome of various interacting factors. The higher the repeatability the greater the confidence one can have that these factors are not fluctuating independently and that the measure is a good one.

ii. Activity in the arena

Ten male and ten female flies were each tested in the arena, the measurement on each fly being carried out three times at intervals of approximately thirty minutes. After each test the fly was removed from the arena with a pooter and introduced into a vial containing food. All the males were tested on one morning and the females on another. This provided a replicate experiment as there is no reason to suppose that the character differs between males and females.

I calculated the variance estimates of the components along with the repeatabilities and these are given in Table 1. The

Table 1

Components of variance and repeatability calculated for activity as measured in the arena.

		Females	Males
Total phenotypic	VP	825	1345
Within flies	VEs	205	177
Between flies	VG + VEg	620	1168
Repeatability		0.75	0.87

Appendix T1.

repeatabilities of 0.87 and 0.75 for males and females respectively are relatively high when compared with those calculated for some morphological and physiological characters in D. melanogaster and other animals (see Falconer, 1960, p. 144). For example in Drosophila melanogaster repeatability for abdominal bristle number is 0.42 and for ovary size, 0.73.

iii. Courtship behaviour

I applied the same method to calculate the repeatability of percentage orientation in courtship. Each male was observed courting three separate virgin females. As the courtship measurements take considerably longer than do those of activity the samples obtained were much smaller. Two samples were examined on different days and, after courtships containing less than twenty units had been discarded, the samples consisted of three and five male flies respectively, each with three separate courtships.

The repeatability and variance components are given in Table 2.

Table 2

Components of variance and repeatability calculated for percentage orientation.

		Sample 1	Sample 2
Total phenotypic	VP	76	310.75
Within males	VEs	15	75.5
Between males	VG + VEg	61	235.25
Repeatability		0.80	0.76

Appendix T2.

Again the repeatabilities are high, being 0.80 and 0.76, and the correspondence between them is very good considering the small sizes of the samples on which the calculations are based.

iv. Discussion

The repeatability of both these behavioural characters is gratifyingly high. These results give one confidence both in the validity of the measures and in the accuracy of the methods employed. They also suggest that the procedure of manipulating the flies does not greatly affect their subsequent behaviour. If there were a large 'disturbance effect' it would probably, due to habituation, get less during repetition of the measure and this would tend to increase VEs. Finally, high repeatability for percentage orientation, which gives a measure of courtship intensity, indicates that the behaviour of normal females from a common environmental background is similar, or, at least, that the fluctuations in behaviour that they do exhibit have comparatively little effect on the courtship of the males. This requires qualification in that a certain number of courtships were discarded as being too short. The statement is true therefore only for the sample of females used. However as my interest was in male courtship and not female receptivity this is not an important drawback. A high proportion of the courtships ended in copulation and it is interesting that neither copulation nor the performance of courtship, at least for the short periods that my tests covered, produced a reduction in the males' tendency to court or in courtship intensity.

3. Genotypic and environmental components of variance

i. Introduction

Measuring repeatability only enables one to separate VEs from the total phenotypic variance and it is possible to make a further and more important division, that between VG and VE. If this is done along with the measurement of repeatability then VEg can be calculated. The separation of genotypic and environmental components of variance is therefore a useful one to attempt.

The method used is based on the comparison in performance between outbred animals and the F1 progeny of crosses between inbred lines. The inbred lines that I used had been subjected to brother-sister matings for over sixty generations. This process should ensure that the flies are homozygous at almost all loci. Theoretically any variance that such flies exhibited would be due to environmental causes. While this is true, inbred flies are considered to be less well buffered against environment fluctuations and thus the variance that they exhibit tends to be greater than VE in an outbred population. The F1 progeny of two inbred lines, however, would also all be genetically similar and would, moreover, be heterozygous at a large number of loci. The variance that they exhibited is therefore more likely to provide a reliable estimate of VE and be more comparable to that of an outbred stock. The variance calculated from an outbred population would be both genetical and environmental in origin (VG + VE = VP) and thus, by subtraction, an estimate of VG and VE can be obtained.

ii. Activity in the arena

I obtained several inbred lines from Dr. F.W. Robertson which had been derived originally from Pacific stock. Two of these were used in the following experiments. Virgin flies were collected from the lines as they hatched and a cross between them was set up in a stock bottle. A stock of outbred Pacific flies was set up at the same time. Progeny from both were collected and tested in the arena. Two separate experiments were done, one using females only and the other males.

The results are given in Table 3. In both males and females

Table 3

A comparison of the performance in the arena between outbred flies and the F1 progeny of a cross between two highly inbred lines.

	Males		Females	
	F1	outbred	F1	outbred
Mean squares entered	35.4	39.3	29.9	25.2
Range	2 - 87	2 - 88	0 - 76	4 - 55
No. of flies	30	30	34	35
Variance estimate	554.5	501.8	319.6	138.9

Appendix T3.

the variance of the outbred flies is smaller than in the F1 hybrids. In other words VE by itself is greater than VG and VE combined and this is clearly absurd. Several examples are known where behavioural characters exhibit variance above the expected values

in certain crosses. Tryon (1940), Scott (1954) and Jakway (1959) have all reported examples in which the variances of behavioural characters were greater in the F1 progeny than in the parent strains. Unfortunately I did not examine the activity levels of the inbred lines from which the Fls that I used were derived but it is probable that the effect I obtained is due to the same cause as in those examples quoted above. Scott, Fuller and King (1959) give the following possible explanation of Scott's (1954) results: 'the F1 hybrids were genetically located near a threshold, so that random environmental factors threw them into one or the other parental type.' If this explanation were applicable to my results I would expect a bimodal distribution of activity levels in the F1 hybrids. Table 4 shows clearly that the distribution is in fact unimodal.

Table 4

The distribution of activity levels exhibited by outbred flies and the F1 progeny of a cross between two inbred lines.

	Number of squares entered								
Number of flies	F1	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90
	Males	5	5	5	5	3	2	2	1	2
	Females	5	6	8	7	4	2	1	1	0
	Total	10	11	13	12	7	4	3	2	2
	Outbred									
	Males	5	1	6	6	2	5	1	2	2
	Females	3	12	9	6	4	1	0	0	0
	Total	8	13	15	12	6	6	1	2	2

Caspari (1958) points out that plasticity of behaviour is often adaptive and considers that the increased variability of heterozygotes over that found in inbred lines could therefore be explained in evolutionary terms as natural selection could establish a threshold at the centre of distribution of a behavioural character. He however limits this argument to the higher vertebrates. It would not in any case be applicable to my results as, on this hypothesis, one would expect the outbred flies also to be maintained at the threshold and thus exhibit even greater variance than the F1 hybrids. Neither of these two hypotheses which have been presented to account for similar phenomena in other animals appear to be applicable to the results of the tests on activity described above.

iii. Courtship behaviour

An attempt to make the same calculation for percentage orientation was made. Unfortunately a high proportion of the F1 males from the same cross used in the previous experiments on activity would not display courtship behaviour at all. As the method is applicable only to a continuously varying character courtship behaviour, in this case showing discontinuous variation, could not be used. There is, however, some evidence that such a method of separation, had it been possible, would have given results as equivocal as those for activity. I examined a series of F1s in an attempt to find a cross that provided less variable courtships than outbred flies. Two of these crosses, I.L. 4♂♂ x I.L. 6♀♀ and I.L. 6♂♂ x I.L. 10♀♀ are strictly comparable

with one another as their courtship behaviour was examined on the same days and their developmental environment similar. The percentage orientation for the flies that did court was calculated along with the variance of this measure. As can be seen from Table 5 although the mean percentage orientation is almost the same in both the Fls, the variance in one, I.L. 4♂♂ x I.L. 6♀♀, is over five times as great as in the other, I.L. 6♂♂ x I.L. 10♀♀. It is obvious therefore that no reliance can be placed on this method of separating VG and VE, at least for the two behavioural measures that I attempted.

Table 5

The variance in percentage orientation in two samples of the F1 progeny of crosses between inbred lines.

	Mean percentage orientation	Variance	No. of courtships
Cross 1	65.1	216.5	15
Cross 2	66.5	37.0	11

Appendix T5.

iv. Discussion

I do not know of any example reported in the literature where there is a similar excess of variance in F1 animals nor does there seem to be any obvious reason for these findings. The method has been used successfully for a number of characters in Drosophila. Ovariole number (Robertson, 1957a) and thorax length (Robertson,

1957b) are two examples. Any attempt at an explanation of these results will have to await a better understanding of the way in which the genotype controls behaviour. There is, however, an assumption implicit in the method which may not always hold true. This is that VE is approximately the same for all genotypes, and, as previously mentioned, this is not always applicable to inbred flies and thus may also not be true of certain other genotypes.

Repeatability may however provide some information on the relative proportions of VG and VE. In the two examples in Drosophila in which both VE and VG along with repeatability have been calculated, that is bristle number (Reeve and Robertson, 1954) and ovariole number (Robertson, 1957a), VEg has been found to be very small in proportion to VEs. While there is not sufficient evidence from which to generalise, it does seem reasonable to suggest, as the environmental conditions during development are kept as uniform as possible, that for both the measures I investigated VG is large. If this were so one would expect that the heritabilities of these characters would be high. Evidence will be presented later to show that, for courtship intensity at least, the heritability is indeed large.

CHAPTER III

The Effects of Some Environmental Factors on Courtship and Activity

1. Introduction

In the foregoing chapter I demonstrated that changes occurring in the environment over a period of a few hours had little effect on two behavioural characters, activity and courtship intensity. The much greater variance in these characters between days and more particularly between generations, is not, from an experimental standpoint, a great hindrance as the measures are all relative to controls and not absolute. Nevertheless these fluctuations, which must be due to some environmental factor or factors, are both disturbing and interesting. The experiments described in this chapter show the results of manipulating some of the obvious environmental factors which might contribute to the fluctuations. I also tested whether courtship behaviour changed throughout the day in a regular and predictable manner.

2. Diurnal Rhythm of Mating Speed

i. Introduction

Diurnal rhythmicity both of physiological and behavioural characters is common in animals. For example, locomotor rhythms have been shown to exist in *Drosophila robusta* by Roberts (1956). In this species the period of greatest activity occurs just before the onset of darkness and this periodicity, once established, continues under conditions of constant illumination and without

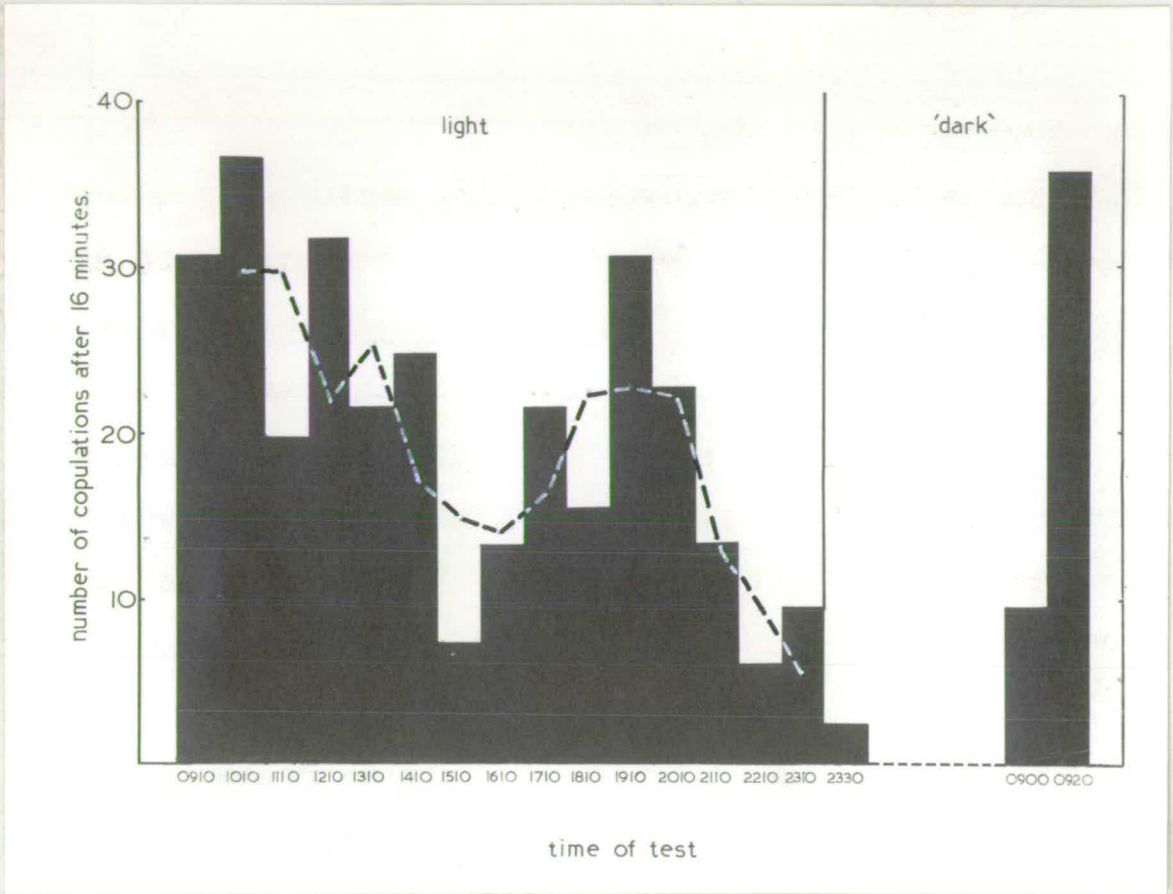
other rhythmic clues from the environment. From this Roberts concludes that the rhythm, once initiated by environmental stimuli, is controlled endogenously. Taylor and Kalmus (1954) found a diurnal rhythm of flight activity in two species of Drosophila, melanogaster and subobscura, in which there were two peaks, one occurring at dawn and the other at dusk.

While the repeatability experiments show that during the period between 9 a.m. and 12.30 a.m. when experiments were normally done the fluctuations in both activity and courtship are not large, it is interesting to see whether sexual behaviour in particular is subject to a daily rhythm over the whole daylight period.

ii. Methods

It is not generally possible to record more than ten single pair courtships per hour. Thus it would be difficult to obtain sufficient figures to demonstrate a diurnal rhythm of courtship intensity using this method, particularly if courtship were poor during part of the day. I therefore used the mass mating technique for these experiments. Mass matings, using fifty pairs of virgin flies each time, were set up at hourly intervals between 0910 hrs. and 2310 hrs. (Dawn and dusk for the flies were at 0900 and 2330 hrs. respectively.) Three further mass matings under conditions of dim red light which was just sufficient for the observer to see the flies, were set up at 2330 hrs., 0900 hrs. and 0920 hrs., the latter two on the morning subsequent to the previous experiments. Bastock (1955) has shown that it is unlikely that the flies can see under such illumination. The flies used in these tests were the

Fig. 6



Diurnal rhythm of mating speed. The dotted line represents a running mean of the figures.

F1 progeny of a cross between two inbred lines which had proved to be less variable in their courtship behaviour than the Pacific stock. The flies were all between three and four days old as were normally used.

iii. Results

The results of these experiments are condensed into Table 6. To demonstrate the change in mating speed throughout the day more clearly I have illustrated, in Fig. 6, the number of pairs that had copulated within sixteen minutes from the start of each test. There is, unfortunately, considerable variability between adjacent tests but the overall trend suggests a bimodal distribution with peaks of sexual activity at approximately 1200 hrs. and at 1930 hrs. The least variable period appears to be between 0900 hrs. and 1400 hrs. It is not surprising to find a bimodal distribution as this correlates with flight activity and it is more reasonable to suppose that there is one 'clock' which controls behaviour generally than that there are several, each with a different rhythm and controlling a different aspect of behaviour.

This rhythm of mating speed also seems to be controlled endogenously as there is a sharp rise in the number of matings between 0900 hrs. and 0920 hrs. on the second morning of the experiment although the light was not switched on at 0900 hrs. in the normal manner and there was thus no external clue.

During the mass matings I watched the general behaviour of the flies in the flasks and the subjective impression that I gained was that the low level of sexual activity in the late afternoon and

Table 6

The results of mass matings set up at intervals throughout the day.

Time of day	Minutes from start of test										
	2	4	6	8	10	12	14	16	18	20	
0910	0	7	13	20	23	24	28	30	32	34	Normal illumination
1010	0	2	9	15	23	30	33	36	39	40	
1110	0	3	5	12	14	17	18	19	20	21	
1210	1	7	13	19	24	28	30	31	33	34	
1310	0	2	6	10	14	18	19	21	24	27	
1410	0	3	9	11	13	16	22	24	28	28	
1510	0	1	1	2	4	6	7	7	7	10	
1610	0	1	3	4	7	8	10	13	15	17	
1710	0	1	3	8	12	14	18	21	24	29	
1810	0	0	2	4	7	8	12	15	17	18	
1910	0	4	10	15	18	20	25	30	32	32	
2010	1	2	3	7	9	13	19	22	24	27	
2110	0	1	3	4	7	8	11	13	14	14	
2210	0	1	2	2	3	5	6	6	6	6	
2310	1	2	3	4	7	7	9	9	9	9	
2330	0	0	0	1	1	2	2	2	2	2	Red light
0900	0	0	1	2	4	6	8	9	9	9	
0920	0	3	9	16	23	29	30	35	37	37	

Number of copulations

evening was due to neither a lowering of the male sex drive or of female receptivity but to a decrease in overall activity. The flies stopped moving about and tended to settle on the walls of the flasks thus decreasing the number of contacts between the flies. Courtship when it did occur seemed quite normal. This observation parallels that of Manning (1963a), who found that males selected for slow mating speed tended to be inactive also. This differs from, but does not contradict, the result of a previous experiment where Manning (1961) has shown that selection for increased mating speed in both males and females results in lowered activity. These results indicate that activity and courtship behaviour are independent but can, nevertheless, influence one another in different ways depending on other circumstances, in this case the technique of selection.

As mentioned in the previous chapter all experiments were normally carried out between 0900 hrs. and 1230 hrs. and the above result indicates that this is probably the best period of the day.

3. The effects of light intensity

While the onset of 'dawn' probably originates the diurnal rhythm of behaviour there is enough evidence to suggest that light intensity has little direct effect on at least courtship behaviour. If Fig. 6 is examined it can be seen that the mass mating carried out at 0920 hrs., in virtual darkness, results in almost as many matings as that at 0910 hrs. on the previous day in normal light. Also it is known that the stimuli concerned in courtship in melanogaster are primarily other than visual in contrast to

species such as D. subobscura and D. auraria which never seem to mate in the dark (Rendel, 1945; Spieth and Hsu, 1950). Manning (1959b) has shown that the courtship success of melanogaster males is scarcely reduced in the dark while simulans males, whose courtship is providing more visual stimuli, have a considerably lowered sexual success under such conditions. Bastock (1955) has watched the courtship of melanogaster under red light and says that it does not differ from courtship in normal light except that if the males lose the females they have more trouble in finding them again. Thus, while there is probably a slight reduction in courtship efficiency in the dark, this is unlikely to be the result of a decrease in sexual drive in the male or receptivity in the female.

There is some evidence, however, that general activity levels might be affected by changes in light intensity. Medioni (1959) showed that the speed of running towards a light source was progressively diminished by blacking out one, two and all three of the dorsal ocelli. Reducing overall light intensity might have the same effect as blacking out the ocelli although Medioni did not test this. The arena is, among other things, a measure of running speed and therefore differences in light intensity could give different measures in the arena.

I examined samples of male flies from the Pacific stock in the arena under two intensities of incident light. These were, measured at the level of the arena, approximately 4300 and 430 foot lamberts. The former was the normal level of illumination used for all measures of activity and of single pair courtships. I tested four samples each on a different day, half the number of

flies in each sample being tested at each illumination. The results are given in Table 7 and do not suggest any consistent difference in activity under these two intensities of illumination.

Table 7

Activity, in the arena, of control flies tested at two intensities of illumination.

	Mean No. of squares entered	Range	p.	more active
Sample 1				
430 foot-lamberts	82.7	63 - 98	< 0.1	at 430 foot-lamberts
4300 foot-lamberts	67.1	16 - 88		
Sample 2				
430 foot-lamberts	47.7	18 - 87	< 0.1	at 4300 foot-lamberts
4300 foot-lamberts	58.4	37 - 76		
Sample 3				
430 foot-lamberts	44.5	1 - 80	< 0.01	at 4300 foot-lamberts
4300 foot-lamberts	68.5	51 - 101		
Sample 4				
430 foot-lamberts	64.3	28 - 94	--	
4300 foot-lamberts	62.0	35 - 90		

Appendix T7.

This appears to conflict with Medioni's result but there are two possible explanations which could account for this. First, the assumption that reducing light intensity and blacking the ocelli are homologous in their action on the nervous system may be wrong. Secondly, Medioni could have been measuring an aspect of activity with few components in common with the arena measure. In the apparatus that he used, a fly was placed in darkness and then a directional beam of light was shone at it. Simultaneously it was stimulated by vibration and the distance and duration of the fly's first run towards the light source measured. This probably lasted for a few seconds only. Thus both the stimulus situation and the time scale of Medioni's experiment differed from mine.

4. Temperature

i. Introduction

Temperature could affect behaviour in both a direct and an indirect manner. Directly, one would expect that behavioural characters in poikilotherms would have a Q10 of the same order as physiological ones. Haldane, for example, has shown that running speed in ants varies with temperature. Indirect effects on behaviour might follow from such findings as that of Apatov (1930) who demonstrated that the temperature during larval and pupal life in melanogaster affects the phenotypic expression of wing size in the adult. This could have repercussions on courtship success as discussed in Chapter V. Another effect of temperature during development is reported by Maynard Smith (1958) who showed that reduction of temperature during larval life resulted in a prolonga-

tion of lifespan in the adult. I have examined the effects of reduced temperature during development and also during testing but did not look at the effect on behaviour of keeping the flies at different temperatures for long periods prior to testing. Maynard Smith has reported that female D. subobscura kept at 30.5°C for five days showed reduced sexual receptivity when returned to 20°C compared with those exposed continually to the latter temperature. Males however subjected to the same procedure did not appear to show any changes in mating behaviour.

ii. The effects of changed temperature during development

Culture bottles with Pacific flies were set up in the normal manner. After the females had been laying eggs for two days the flies were discarded and the culture bottles placed in a constant temperature box which was kept at 18 ± 1°C. The development of the flies at this temperature takes about thirty days and therefore cultures at 26°C were set up later to ensure simultaneous hatching of the flies reared at the two temperatures. The flies were all aged at 26°C and tested at this temperature as usual.

In order to measure the increase in wing area due to rearing flies at 18°C I removed one wing from ten males in each sample. These were mounted on microscope slides and, by using a camera lucida, I drew the outline of the wings on squared paper. The areas of the wings were then calculated and are given in Table 8. There is no overlap between the two samples and the mean increase in wing area of flies reared at 18°C over those reared at 26°C is almost 29 per cent.

Table 8

The wing areas of flies reared at 18°C and at 26°C. The unit of measurement is an arbitrary one.

Reared at 26°C 1787 ± 24

Reared at 18°C 2300 ± 25

This represents an increase in wing area of 28.7 per cent in the flies reared at 18°C.

Activity in the arena

Two samples of male flies from each category were tested in the arena and the results of these measures are given in Table 9. There is no consistent difference between the activity of flies reared at 18°C and at 26°C when tested at the latter temperature.

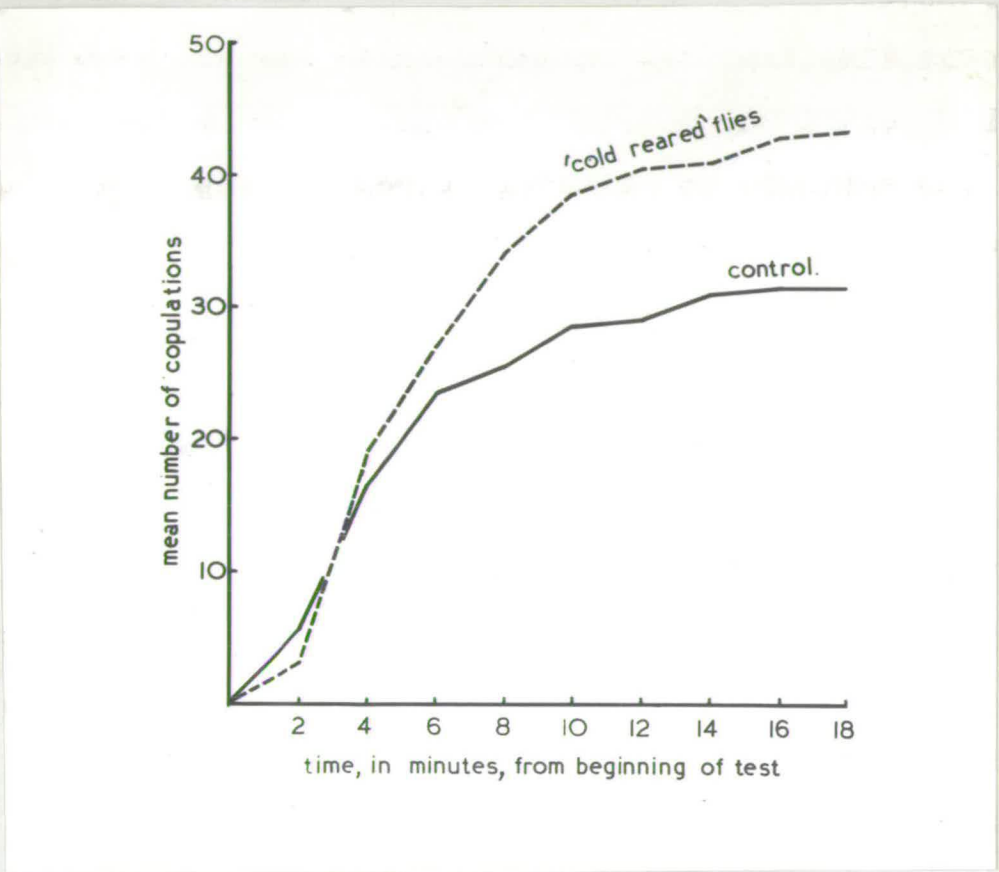
Table 9

Activity, in the arena, of flies reared at 18°C and at 26°C.

	Mean No. of squares entered	Range	p.
Sample 1			
18°C	69.6	1 - 99	< 0.1
26°C	85.2	53 - 112	
Sample 2			
18°C	76.0	29 - 102	-
26°C	76.2	53 - 95	

Appendix T9.

Fig. 7



Mass mating graphs of males reared at 18°C compared with controls reared at 26°C.

Appendix F7.

Mass Matings

Mass matings were set up in which males reared at 26°C and at 18°C were tested with control stock females reared normally. Two separate experiments were carried out, fifty males of each type being used each time, and the summed results are illustrated in Fig. 7. This result suggests that males reared in the cold mate faster than the controls or, in other words, they are more successful with females than controls. The hypothesis that I suggest to explain this increased mating success is discussed fully in Chapter V along with further evidence in support of it. Briefly it is that, by virtue of their greater wing area, the flies reared at 18°C provide a greater amount of sexual stimulation to the females, via vibration, than do the controls. This presupposes, among other things, that the amount of vibration provided by males from the two samples is the same. To check this I examined ten single pair courtships from each class of male. The means of the three courtship elements is given in Table 10 and it is evident that rearing flies at 18°C has no significant effect on the courtship intensity of the males, although the effectiveness of their courtship is increased.

Table 10

The percentage of courtship elements exhibited by [redacted] males reared at 18°C and 26°C courting control females.

males reared at:	pefcentage prientation	percentage vibration	percentage licking
18°C	58.60	28.54	12.86
26°C	57.37	31.36	11.27

Appendix T10.

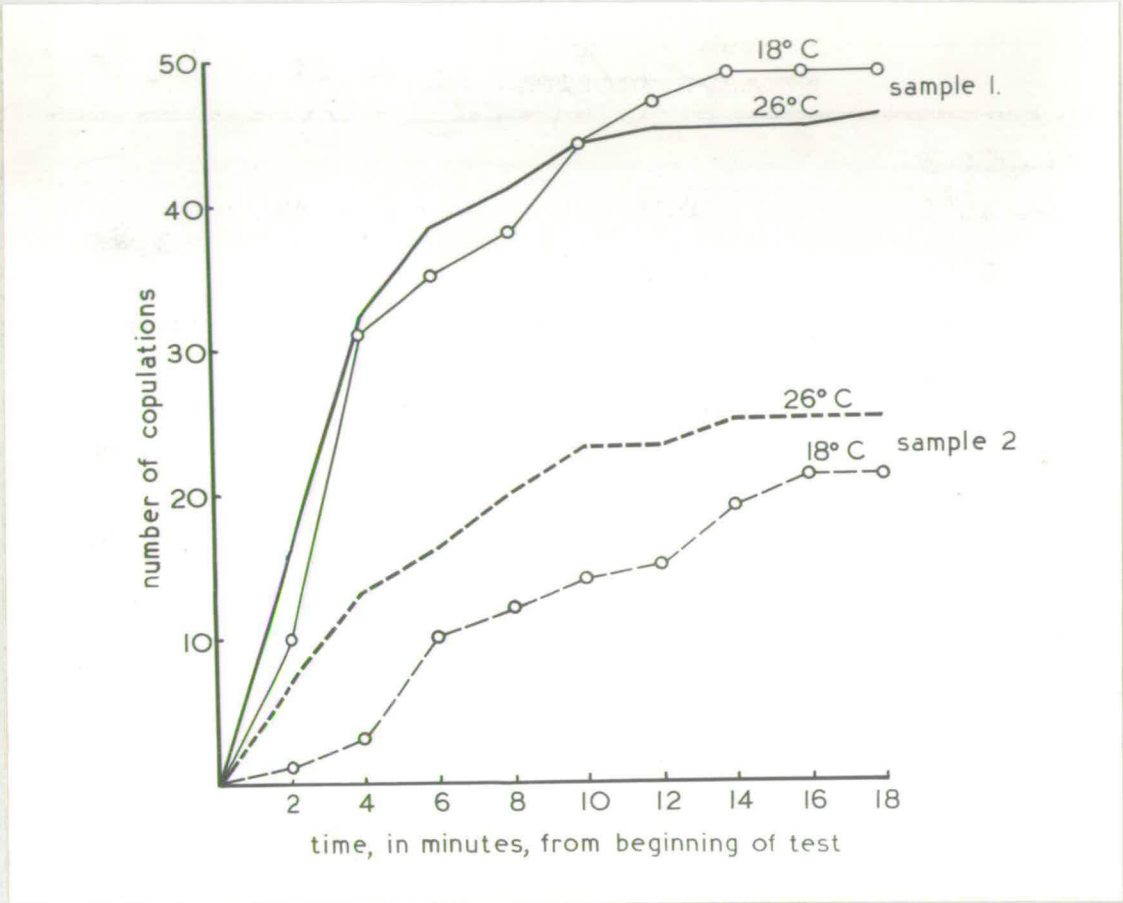
iii. The direct effects of temperature

Activity in the arena

I examined the activity, in the arena, of samples of male Pacific flies at 26°C and at 16°C. All the environmental factors except temperature were made the same as possible for the two samples. I left the flies to be tested at 16°C at this temperature for at least 30 minutes to allow them to equilibrate to this temperature before testing although they were reared and aged at 26°C as usual.

I tested two samples on different days with 25 flies at each temperature on both occasions. The results are given on Table 11 and from these I calculated Q10s for each sample. These were 1.75 and 1.69 respectively. Q10s for most biological systems that have been examined are between 2 and 3 thus resembling the values for chemical rather than physical processes (Heilbrunn, 1952). The low values obtained for activity could be due to two causes. First, their values are compatible with the Q10 for the rate of conduction along nerve fibres and it could be that the temperature is affecting the rate of discharge of nerve impulses from centres controlling activity. Second, it is known that large insects, if the ambient temperature is not sufficiently high, can raise the temperature of their flight muscles by vibrating their wings prior to flight. Chadwick (1939), however, has demonstrated that Drosophila, due to its small size, equilibrates very quickly with the temperature of the surrounding air even during flight. Therefore while it is possible that the low Q10 for activity is due to the flies compensating for the lowered temperature in a comparable way, this explanation is unlikely.

Fig. 8



Mass matings carried out at 16°C and 26°C, showing large between day variance. The two samples were tested 48 hours apart.

Table 11

Activity, in the arena, of control flies measured at 16°C and at 26°C.

	Mean No. of squares entered	Range	Q 10
Sample 1			
at 16°C	30.8	1 - 60	1.69
at 26°C	52.1	11 - 90	
Sample 2			
at 16°C	19.4	1 - 43	1.75
at 26°C	34.0	2 - 63	

Appendix T11.

Mass matings

I tested the mass mating speed of stock flies at 18°C and at 26°C. The experiment was set up twice using, as usual, fifty pairs of flies on each occasion. As in the activity experiments the flies tested at the lower temperature were allowed to equilibrate to it for 30 minutes.

The values for these tests are illustrated in Fig. 8. One test shows the expected result in that mating speed is slower at 18°C than at 26°C. In the other, however, no such conclusion can be drawn. Further mass matings at these two temperatures would clarify the results described but the importance of these two mass matings is that a far greater difference is found between the two samples than within them. Thus the difference due to some uncontrolled environmental factor is larger than that caused by the experimental change.

Fig. 9

Differences in the pupation sites of larvae with a common genetical background and reared under similar environmental conditions.

5. Nutrition

It has been assumed that the medium obtained for culturing the flies was reasonably standard and did not vary much between batches. Even if the bottles and vials were identical when the flies were introduced it is unlikely that the micro-environment of different bottles changes in an identical way during the development of the larvae or ageing of the flies. It is possible that relatively small changes in, for example, the concentrations of excretory products or of alcohol or carbon dioxide in the food could have quite large effects on the subsequent behaviour of the flies. That some differences between culture bottles can develop is shown by the photograph in Fig. 9. This shows two bottles in which the amount and quality of the food was almost the same. The number of control flies and the length of time that they were allowed to lay eggs were the same in both as were the external environmental factors. However the pupating behaviour of the larvae was quite different in the two bottles. I do not know the reason for this difference but if such contrasts in larval behaviour exist then it is also possible that correspondingly large changes can also occur in the adults.

I carried out one experiment in which I examined the effect, on courtship, of starving the larvae. It is known from a variety of insects, and other animals, that crowding the larvae so that there is competition for food reduces body size in the adults. I set up two series of vials. In each of the first I introduced one pair of flies, allowed them to mate and lay eggs for one day and then discarded them. In each of the second were 15 pairs of

flies which were allowed to lay for three days. Male progeny obtained from the latter crowded conditions were, on the average, 9.6 per cent smaller in thorax length than the uncrowded flies and there was no overlap between the two classes with respect to this measure. The flies from the crowded conditions appeared perfectly normal and in no way sickly and it seems likely that under natural conditions competition for food would be the usual situation rather than an overabundance of food. The experiment was done twice and the courtship of ten males from each class examined each time. These results are shown in Table 12. The courtship intensity of the 'environmentally' small males is significantly lower* than that of the controls but this change is not nearly as great as is found, for example, between the lines selected for changed body size which are described in Chapter IV.

Table 12

The percentages of courtship elements exhibited by 'environmentally' small males compared with control males. Control females were used throughout.

	percentage orientation	percentage vibration	percentage licking
'Environmentally' small males	62.65	26.75	10.60
Control males	54.75	34.60	10.65

Appendix T12.

An example of the type of change that can occur over a short period of time is illustrated in Fig. 8. This shows two mass

* $p < 0.001$.

mating experiments set up at two days interval. The results of the experiment have already been discussed earlier in this chapter. The two samples were cultured in bottles from the same batch and the parents of the flies were the same. The only known differences between the samples are that the flies in sample 2 are derived from eggs laid two days later than those in sample 1 and the food had been kept in a refrigerator for two days more.

6. Discussion

While these experiments are far from comprehensive they do show that fairly large changes in certain obvious environmental factors have relatively little effect on the measures of behaviour that I employed compared with some unknown factor or factors. This is best demonstrated in Fig. 8 where the within sample difference in mating speed due to a difference of 8°C is several times less than that between the samples which must have been caused by some change in the environmental conditions either during development or during the actual testing.

Light intensity seems to have no effect on either activity or on mating speed, at least within the range that I looked at. Changes in temperature both during development and while experiments were being carried out did affect the flies' behaviour but in a predictable manner. The magnitude of these effects show that they could not, by themselves, account for the large differences often found between days and between generations. There seem to be three possible sources of these fluctuations.

1. They could be caused by some environmental factor which was

not taken into account or controlled. One possibility is vibration from a neighbouring workshop. This, however, is unlikely as the experiments in which flies were tested at different temperatures were carried out in rooms on different floors of the building. The difference in activity due to temperature remains the same in both samples while the overall level of activity is considerably lower in the second sample.

2. Two or more environmental factors, while not by themselves having a large effect on behaviour over their normal ranges, could, in conjunction, have more than merely an additive effect. Such interactions would be very complex and difficult to test.

3. The most likely possibility, as previously mentioned, seems to be that a small change in the concentration of some constituent of the medium could, by affecting the threshold of the behavioural characters, have a disproportionately large effect on the behaviour. Possibly this could be tested by examining flies reared in a sterile and strictly characterised medium.

The experiments described in this chapter emphasize the necessity of obtaining repeatable and consistent measures of any differences that are thought to exist. Often significant differences are found between samples as for example in the activity measurements at the two light intensities. In this case the difference was neither repeatable nor consistent and was therefore due to some uncontrolled factor in the environment. Fortunately the measures are usually more reliable than in this instance and the results of experiments on courtship behaviour are less prone to this type of fluctuation.

CHAPTER IV

The Effects of Changed Body Size

1. Introduction

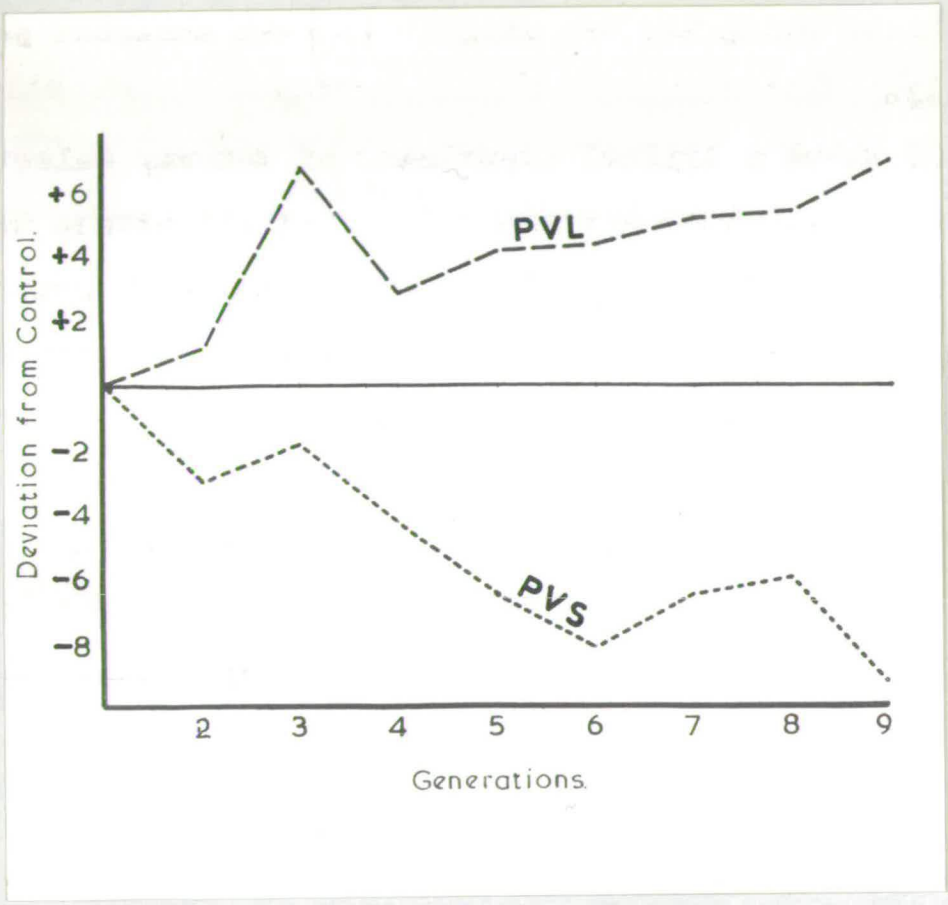
In the introduction to this thesis I listed the main ways in which it is possible to utilise genetical techniques for behaviour studies. I have already described some experiments in which the methods of quantitative genetics were applied to two behavioural characters. In this chapter is described the behavioural analysis of several lines of flies that had been subjected to a two-way selection for a morphological character, namely body size. I was encouraged to examine these lines because of the observation, by Dr F.W. Robertson, that there seemed to be differences between them with respect to mating speed.

2. Materials and Methods

I obtained from Dr F.W. Robertson four lines of flies of which two, PAL and PBL, had been selected for large, and two, PAS and PBS, for small body size. All the initial experiments were carried out on these lines. Two further lines, PPS and PPL, were obtained later and these had also been selected for small and large body size respectively. I checked that the behavioural differences in the initial lines were also present in these lines and used them in certain of the experiments.

The criterion used for selection was thorax length rather than overall body size as it is more easily measured and is positively correlated with total body size. I continued selection using the

Fig. 10



The results of two-way selection for body size. The response is expressed as percentage deviation from control with respect to thorax length.

same technique as Dr F.W. Robertson. The thorax lengths of 100 pairs of flies from each line were measured each generation using a microscope with a micrometer eye piece and the large and small lines continued using the ten largest and ten smallest pairs respectively.

Fig. 10 shows a typical experiment of two-way selection from a base stock. Although the graph does not illustrate the final result, a plateau is reached after approximately 25 generations when the increase in thorax length is about 10 per cent and the decrease 13 per cent. A more complete description of the selection methods and a discussion of the genetical results of similar selection experiments is given by Robertson and Reeve (1952) and Robertson (1955). The mean divergences of thorax length from control over the five generations that the experiments covered are given in Table 13.

Table 13

Generation	PAL		PBL		PAS		PBS		Control	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
9	102.2	117.9	100.6	116.8	88.0	100.6	91.6	104.3	95.1	110.9
10	102.4	118.0	101.6	117.4	not measured		90.2	103.2	95.1	110.3
11	100.0	116.8	100.0	116.9	85.8	101.1	87.1	102.4	93.5	107.3
12	101.3	118.2	100.7	116.8	85.2	99.2	85.8	100.5	93.3	108.6
13	102.9	119.8	100.2	116.8	85.5	100.8	88.0	101.4	93.7	110.9

The figures refer to the mean thorax lengths of the F1 progeny of selected parents, measured in hundredths of a millimetre.

3. Courtship Behaviour of the Selected Lines

The courtship behaviour of 20 males from each line and from control was recorded while they were courting control females. Table 14 shows the mean percentages of the three courtship elements for each of the lines and control. A small sample of males from each line was also examined courting their own females in order to eliminate the possibility that the differences obtained were due to a differential response of the selected males to the control females. As can be seen from Table 15 approximately the same order of difference is found under these conditions thus ruling out this possibility.

PAL and PBL males display significantly less vibration than do PAS and PBS males while the controls are intermediate between the large and small flies. Thus, along with a two-way divergence in body size, there is a parallel divergence of courtship intensity. When the PPL and PPS lines were obtained their courtship was examined and a similar divergence in courtship behaviour was found to have occurred. Thus in three replicate pairs of lines selected for extremes of body size reduced vibration has been found to accompany increase in body size and increased vibration, decrease in body size. The remainder of this chapter describes an attempt to analyse the reasons for this divergence. I considered the following possibilities.

a. There could be a straightforward connection between phenotypic body size and courtship behaviour. Change in body size, regardless of its cause, would result in the type of courtship divergence found in the selected lines.

Table 14

The mean percentages of the three courtship elements in the lines selected for large and small body size.

Line	% orientation	% vibration	% licking
PAL	70.5	24.5	5.0
PBL	63.45	30.15	6.4
Control	53.45	37.0	9.55
PAS	40.35	51.9	7.75
PBS	39.7	49.05	11.25

Appendix T14.

Table 15

The mean percentages of the three courtship elements in the selected lines where the males are courting their 'own' females.

Line	% orientation	% vibration	% licking
PAL	67.6	26.6	5.8
PBL	61.7	31.0	7.3
PAS	30.9	58.1	11.0
PBS	44.0	45.0	11.0

Appendix T15.

b. The genes controlling body size, or linked genes, might have a pleiotropic effect on courtship behaviour.

c. The lines might have changed in some general metabolic or physiological way. For example reduction in metabolic efficiency due, perhaps, to an increase in body size could lower both courtship intensity and the level of general activity. Thus, an examination of the activity levels could indicate whether this were so.

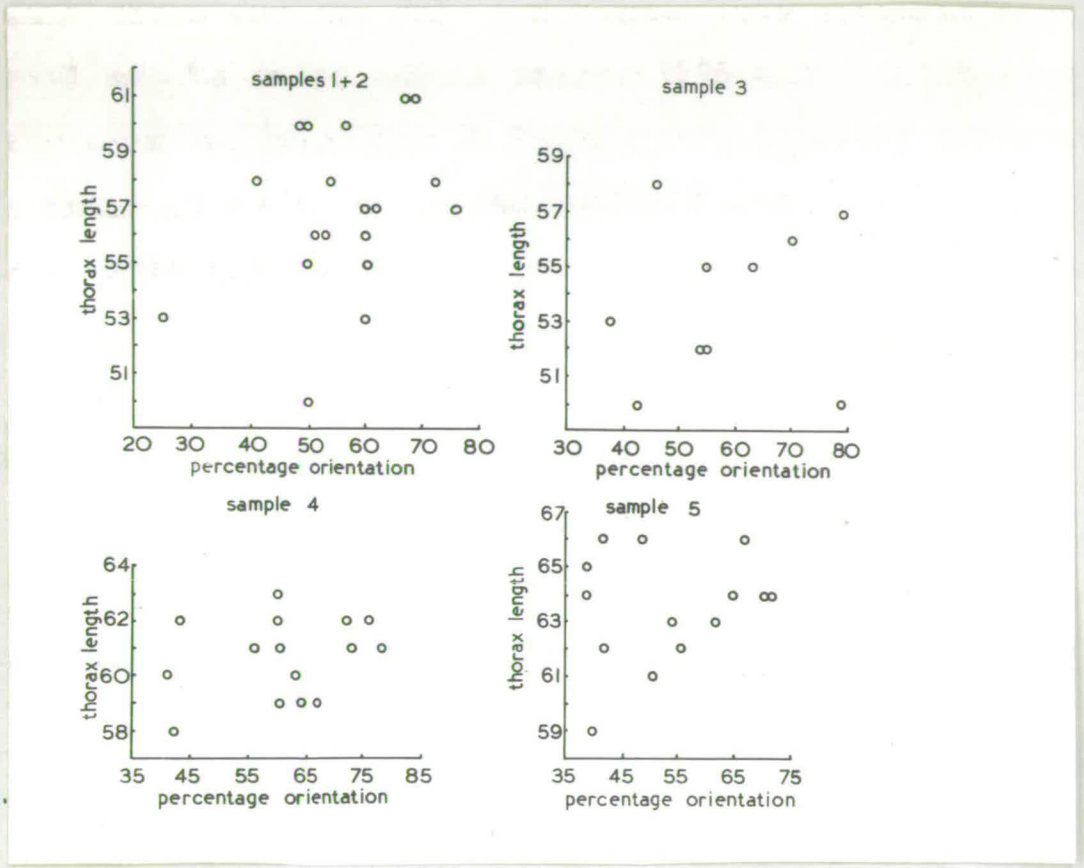
d. Change in body size could affect courtship efficiency and thus create a secondary selection pressure for changed male courtship.

4. Phenotypic Body Size and Courtship Behaviour

Although it is not possible to increase body size, by environmental changes, beyond the limits found in the control stocks the opposite is possible. Size can be reduced by rearing the larvae under crowded conditions such that there is severe competition for food. The results of this experiment have already been reported in the previous chapter. The courtship of these 'environmentally' small males resembled not that of the small selected lines but that of the large males, exhibiting less vibration than the controls.

Although the variability in body size in the control stocks does not embrace the limits found in the selected lines or in the 'environmentally' small flies there is inevitably some variability and this could be sufficient to demonstrate a connection between phenotypic body size and courtship behaviour. I therefore examined the courtship of 58 control males and subsequently measured their

Fig. 11



Thorax length plotted against percentage orientation in courtship for four samples of control male flies.

thorax lengths. These courtships were examined in five separate samples and the mean percentage orientation and mean thorax length in each is given in Table 16. Samples 1 and 2 were lumped as the means of both measures were almost the same and the other three treated separately. The differences in the means of the thorax lengths do not reflect differences in actual size but merely that I had not at this time standardised my method of measurement and had used microscopes with varying magnifications for some of the samples.

Table 16

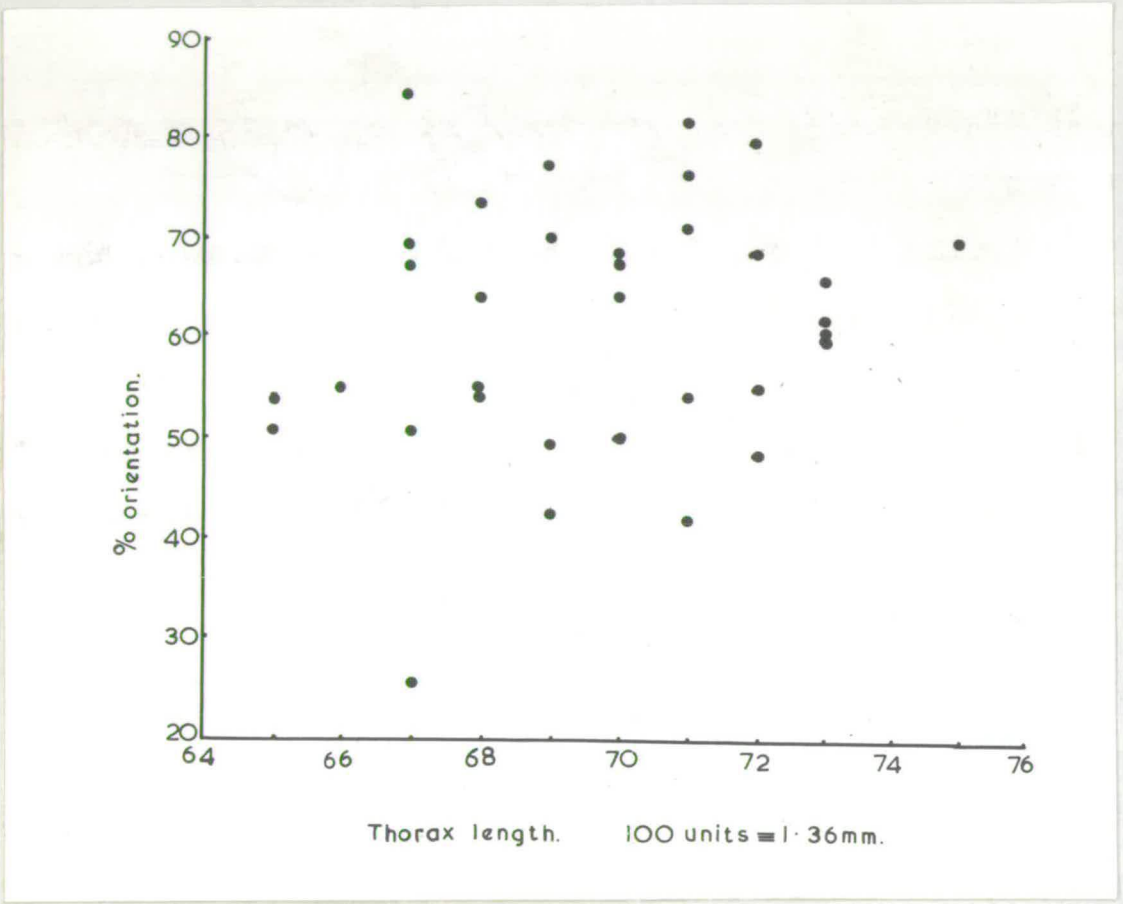
Thorax length and percentage orientation in five samples of control flies. Thorax length was measured on an arbitrary scale.

	Number of courtships	Mean thorax length	Mean % orientation
1	9	57.0	56.4
2	10	56.8	56.1
3	10	53.8	58.2
4	15	60.7	61.0
5	14	63.5	53.4

Appendix T16.

A connection between phenotypic body size and courtship intensity of the type found in the selected lines would give a positive correlation between thorax length and percentage orientation. I therefore plotted these values for each of the samples and the graphs obtained are illustrated in Fig. 11. I also calculated

Fig. 12



Thorax length plotted against percentage orientation in courtship for the male F2 progeny of crosses between the large and small selected lines.

Appendix F12.

F values for each set of figures which show that there is no evidence for any such trend in any of the samples. From these experiments it would seem that there is no straightforward phenotypic connection between courtship and body size.

5. The pleiotropic effect on courtship of the genes controlling body size

The following experiment was carried out to examine the second possibility listed, namely that the genes controlling body size (or genes closely linked to them) are having a pleiotropic effect on courtship behaviour. At the same time this experiment is a further test as to whether body size and courtship behaviour are phenotypically connected.

The courtship of 34 male F2 progeny from crosses between the large (PAL and PBL) and small (PAS and PBS) lines was recorded and the percentage orientation calculated for each male. As in the previous experiment their thorax lengths were measured and plotted against percentage orientation.

Independent reassortment of genes in the F2 generation results in variation in thorax length that almost includes the range in size between the large and small selected lines. The courtship also varies between the extremes found in the selected lines. If courtship is being influenced by the same genes that are controlling body size then the two measures should be related in the F2 progeny. Fig. 12 shows the result of plotting thorax length against percentage orientation. A regression analysis gave an F value of 1.08 suggesting no degree of correlation. It therefore seems unlikely

that the divergences in courtship behaviour are caused by the pleiotropic effects of the genes controlling body size. It also shows that increased orientation is not a consequence of increase in body size. This possibility could not be taken into account in the previous section on phenotypic body size and courtship behaviour, due to the impossibility of increasing body size much above the control limits without employing selection techniques.

6. Courtship Behaviour and Activity

In the unselected stocks, natural selection maintains body size over a small optimum range and selection away from this optimum would be expected to affect other characters such as physiological efficiency or metabolic rate. If these characters were displaced from their optimal values there could be purely passive repercussions on courtship behaviour. An example of this is the deficient courtship exhibited by inbred D. subobscura. Maynard Smith (1956) attributes this to a lowering of 'athletic ability' in the inbred males. A general measure of activity would almost certainly reflect changes in such a character along with any physiological or metabolic changes and this was therefore measured in the four selected lines and control.

Unfortunately I had not made the arena for measuring activity at this time and the sector apparatus was used. The activity of 50 males and 50 females from each selected line and from control was measured and the results are given in Table 17. Analysis of A χ^2 variance showed that all the selected lines were less active than controls. There are also differences between the lines and PSA is

less active than the other selected lines. These differences in activity do not correlate with differences in either courtship behaviour or body size. It is possible that with the large males their sluggishness and low intensity courtship may be connected but this is not applicable to the small males, which, in spite of being less active than controls, nevertheless exhibit higher intensity courtship. A reasonable suggestion would be that activity is held at a high level in the wild type population and any process of artificial selection would tend to depress activity. However the results of experiments to be described in the following chapters show that this is not a safe generalisation to make.

Table 17

Lines compared	χ^2	Significance	More active line
Control & PAL	38.0	< 0.1%	Control
Control & PBL	7.1	< 1 %	Control
Control & PAS	12.9	< 0.1%	Control
Control & PBS	5.3	< 5 %	Control
PAL & PBL	14.6	< 0.1%	PBL
PAL & PAS	8.6	< 1 %	PAS
PAL & PBS	19.7	< 0.1%	PBS
PBL & PBS	0.1	-	
PBL & PAS	0.8	-	
PAS & PBS	2.0	-	

Appendix T17.

7. Selection effect, I

The previous experiments indicate that the divergences in courtship behaviour in the selected lines cannot be attributed to any straightforward correlate of body size. Therefore the fourth possibility, namely that there had been secondary selection for changed courtship, was examined.

Little is known of the quantitative aspects of stimulation in the courtship of D. melanogaster. As long ago as 1915 Sturtevant showed that removal of the wings from males results in a substantial increase in courtship time. The courtship of flies with large wings induced by subjecting the pre-adult stages of the flies to cold has already been shown to be more stimulating to the females. It is, therefore, possible to conceive that increase or decrease in body size, and correspondingly of wing area, could affect the quantitative aspects of sexual stimulation. One measure in which this would be reflected is the relative mating success of males from the various selected lines. To test this the following 'female choice' experiment was carried out. The males of one large (PAL), one small (PPS) line and control were compared for mating success. The comparisons were made by pairs, PAL males being compared with PPL males, PAL with control and PPS with control. Five males at a time from each of the pair of lines being tested were placed in a 3 x 1 inc vial along with five females from one of the lines and the copulations scored. This was repeated until approximately 45 copulations were obtained in each class. To ensure that the scoring was done correctly the wings of one line in each pair was marked and this procedure was randomised throughout the experiments to eliminate any

bias due to the marking.

Table 18 shows the results of these experiments. The main fact that emerges is that the small males are less successful than either the control or the large males. Also interesting is the tendency of the small males to be more successful with their own than with 'foreign' females. This suggests that the relative sizes of male and female are important.

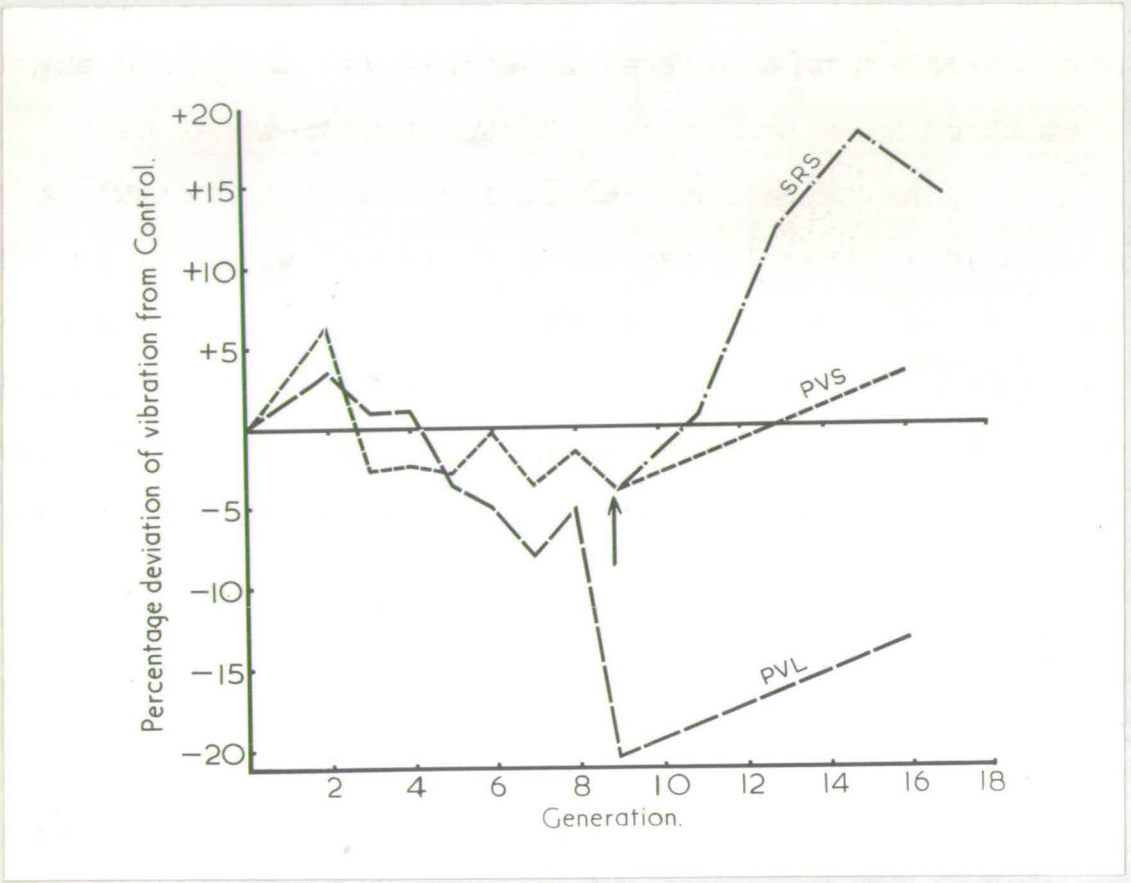
Table 18

The results of the female choice experiments.

Female	Competing males	No. of copulations	χ^2	Significance
PPS	PPS PAC	17 25	8.05	< 1.0%
PAC	PPS PAC	12 30		
PPS	PPS PAL	23 24	4.25	< 5.0%
PAL	PPS PAL	13 37		
PAC	PAC PAL	20 23	-	-
PAL	PAC PAL	23 19		

The design of the choice experiment is such that the difference in the amounts of vibration exhibited by males tends to be reduced in importance. In the confined space of the vials males frequently change from courting one female to another and the males with little vibration benefit from those that show more. This has previously

Fig. 13



Percentage deviation of vibration in courtship from control on flies subjected to two-way selection for body size but with sexual competition between males eliminated (PVL, PVS). Competition (indicated by arrow) introduced at generation 9 in the small line results in increased vibration (SRS).

Appendix F13.

been shown by Sturtevant (1915) who demonstrated that the sexual success of wingless males is increased if they are mixed with normal wild type males. The success of the wingless males in this situation did not, however, reach the same level as that of winged males. The female choice experiment that I carried out therefore suggests that the small selected males' courtship is deficient, not necessarily only in vibration, but in some other components as well.

The reduced success of the small males allows us to put forward a hypothesis to explain why they exhibit more vibration than the control males. It is possible that, within the small lines, there has been competition between males and thus selection for increased vibration which would tend to compensate for the otherwise deficient courtship.

8. Selection effect, II

To test this hypothesis I selected flies for large and small body size in exactly the same manner as before but instead of placing all ten selected pairs of each line in stock bottles each pair was introduced separately into a vial with culture medium. Under these conditions there could be no sexual competition and consequent selection pressure for increased vibration. The results of this selection on body size are shown in Fig. 10.

The courtship of fifteen males from the small, PVS, large, PVL, and control lines thus set up was examined each generation. Fig. 13 is a graph of generation plotted against deviation of percentage vibration from control. On the hypothesis presented it would be expected that there would be no deviation from control in the amount

of vibration in the small males' courtship and this is indeed so.

The elimination of sexual competition between small selected males has also eliminated the divergence in courtship behaviour. The PVL males' courtship has diverged in the same manner as in the original large selected lines (PAL, PBL, PPL), the regression of PVL on control for percentage vibration being significant at the 1% level. This is also what would be expected. There was no difference in the mating success of the large selected lines and control and therefore no reason to suspect that the divergence in courtship found in these lines was due to secondary selection.

I also measured the activity of these lines, this time in the arena, and the results are given in Table 19. As was found in the original large selected lines the PVL flies were less active than the controls. The PVS flies did not, however, differ in their activity level from the control. If there were any connection between courtship behaviour and activity in these lines it was certainly not an obvious one.

Table 19

The activity of PVL, PVS and control flies measured in the arena.

	PVS	PVL	Control
Mean squares entered	56.6	40.8	53.2
Range	15 - 89	1 - 82	5 - 90
No. of flies	40	40	40

Appendix T19.

If the hypothesis presented above to explain the divergence in courtship behaviour in the original small selected lines is correct one would predict that the reintroduction of sexual competition in the PVS line would result, once again, in increased vibration in the males. I therefore tested this in the following way.

Ten pairs of small, PVS, flies were placed in a half pint culture bottle and selection for small size was continued under mass mating conditions as in the original small selected lines. As a control ten pairs of control stock flies, which had been continued in the same way as PVL and PVS lines, one pair per vial, were also transferred to a culture bottle.

The courtship of approximately ten males from each was then examined on alternate generations. Fig. 13 also shows the result of this experiment. This small line, SRS, was initiated on generation 9 and the percentage vibration after a few generations has increased so that the courtship of this line now resembles that of the original small lines. Therefore the reintroduction of sexual competition has been followed by a significant increase in percentage vibration. As an additional precaution the PVL, PVS and their control line was continued throughout the period of this experiment although selection was relaxed. Their courtship was examined near the end of the experiment and Fig. 13 shows that their courtship remains virtually unchanged.

9. Conclusions

One of the interesting points that emerges from these experiments is that seemingly comparable changes in courtship in the small and

large selected lines on analysis proved to be the result of different causes. In the small selected lines the pattern of courtship behaviour was apparently changed by the action of a secondary selection pressure conferring an advantage on flies that exhibited a high percentage of vibration in their courtship. This selection pressure arose as the result of decreased mating success following reduction in body size. The reason for this reduction in success may be that the small flies, by virtue of their reduced size and wing area, are less stimulating to the females. Inbreeding may also have a deleterious effect on courtship success as Smith (1956) has shown for D. subobscura and one of the consequences of selection for small body size is that the flies become inbred and this does not happen during selection in the opposite direction. Thus back selection starting on generation five is effective in returning both large and small lines to the control level while a similar back selection twenty generations later succeeded in the large line but had no effect on the small (Robertson, 1955).

In the large selected lines the reduced levels of activity and of courtship intensity may both be a direct consequence of selection for increased body size as both these changes occurred in all the large selected lines. However, as already mentioned, these two measures are not necessarily correlated. It is not possible therefore to suggest that the reduced courtship intensity in the large selected lines is due to some factor such as reduced metabolic rate or efficiency without further information, although this possibility remains.

While I do not have a hypothesis to explain the reduced

courtship intensity found in the large selected lines, a comparable argument to the one used above would explain why the large males, although exhibiting less of the sexually stimulating element, vibration, are equally successful in obtaining mates as the controls. Their increased body size and wing area would tend to compensate for this lowering of courtship intensity. One experiment which suggests that this explanation is reasonable has already been described in Chapter III and the next chapter deals with further experiments on the quantitative aspect of sexual stimulation.

CHAPTER V

The Role of Vibration in Courtship

1. Introduction

Sexual arousal in melanogaster males is far more rapid than in females and a normal male will often begin courting and attempt to copulate almost immediately he becomes aware of the female. This quick rise in sexual excitation in the male has led Milani (1956) and Hoeningsberg and Santibanez (1959) to conclude that there are two separate patterns of male courtship. The first is '"simple" comprising few elementary actions in an orderly sequence' and the second, '"elaborated" to include quite distinct additional elements in no fixed order' (Milani, 1956). A more likely interpretation is that the male on first meeting the female receives from her only positive sexual stimuli and this results in a burst of courtship on the part of the male which is not inhibited by any action from the female. Thus the sequence orientation, vibration, licking and attempted copulation takes place in a few seconds. This first attempt is rarely successful as few females are receptive enough initially and the female then makes repelling movements that have a mild inhibitory effect on the male. Courtship then settles down to the type of fluctuating pattern that has already been described. Thus it is the female that plays the major role in discriminating against foreign or otherwise unsuitable mates and she requires a certain amount of specific stimuli before she is prepared to mate.

An assumption implicit in the above paragraph is that during courtship the stimuli from the male have an additive effect in

stimulating the female. Although it is not usually possible to demonstrate the quantitative aspect of courtship stimulation in animals, with melanogaster, this can be done. It is known that a major part of the sexual stimulation is provided by vibration. Wingless males, although females will eventually accept them in most cases, are much less successful than normal, winged males (Sturtevant, 1915). That the stimuli from vibration are received via the females' antennae has been demonstrated by Mayr (1950) and Petit (1958). The latter worker supplies evidence which suggests that the nature of this stimulation is not olfactory but due to the physical effects of the vibrations themselves. The amount of stimulation provided by the male from vibration will therefore depend on the following factors.

i. The distance between the male and female during vibration.
ii. The amplitude of the wing beat.
iii. The frequency of wing beat.
iv. Wing area.
v. The proportion of courtship that consists of vibration.

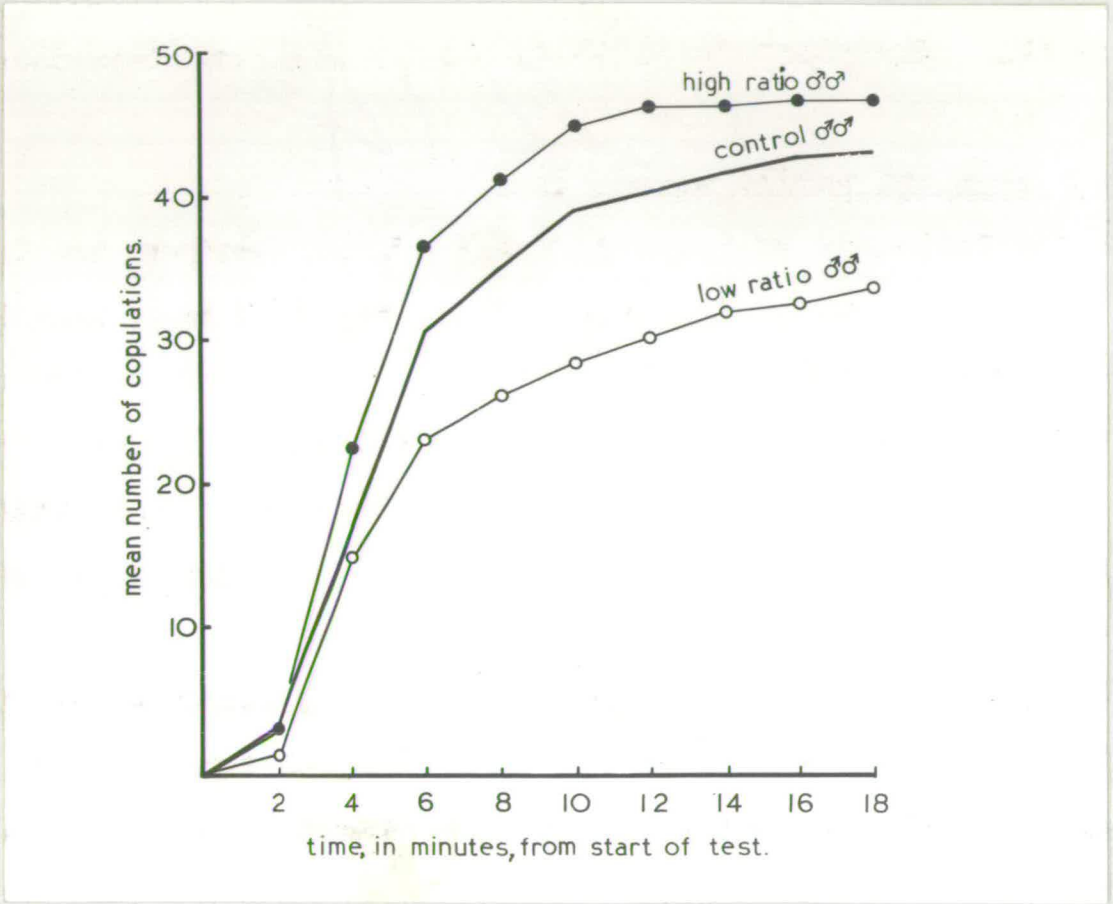
The first three factors are not easily measured and I had to assume that they were constant between males. These assumptions are probably justified on the following grounds. Males usually follow as closely behind the females as possible during courtship and therefore the distance between the sexes will vary within similar limits in the courtship of flies from the same stock. The inability of inbred or weak flies to keep up with the females could reduce the effectiveness of such males' vibration although I have no evidence of this.

It is possible to measure both the amplitude and frequency of wing beat during flight. However, the methods used normally require that the flies are tethered and while flight can be induced under such conditions it is unlikely that the males would court. What evidence there is from studies on Drosophila flight shows that increase in wing area results in decreased amplitude and frequency of wing beat (Reed, Williams and Chadwick, 1942). If this is applicable to courtship vibration one would expect that the increased stimulation provided by flies with larger wings would be to some extent counteracted by decreased amplitude and frequency of the wing beat. However it has been shown by Shorey (1962) that the frequency of wing beat during vibration at 28°C is between 30 and 35 cycles per second while during flight the rate is much higher at about 200 cycles per second. This would suggest that flight and vibration are not controlled in the same manner and that conclusions from flight cannot necessarily be applied to vibration.

The one variable that can be easily changed is wing area and there are several ways in which this can be done. If the effects of changing wing area are examined, however, it is necessary to ensure that the proportion of vibration in courtship remains constant between courtships as this factor has been shown to affect courtship success. Bastock (1956) has demonstrated that males carrying the mutant gene 'yellow' are sexually less successful than the wild type males and that this is attributable to the 'yellow' males' courtship containing shorter and more widely spaced bouts of vibration than that of the wild type.

I examined the courtship success of males with different sizes

Fig. 14



Mass mating graphs of males selected for high and low ratios of wing length to body size respectively compared with that of controls.

Appendix F14.

of wings in three ways. One experiment in which I showed that males with environmentally induced large wings had a faster mating speed than the normal controls, while the percentages of the courtship elements remained constant, has already been described in Chapter II. The other two methods that I used are described below.

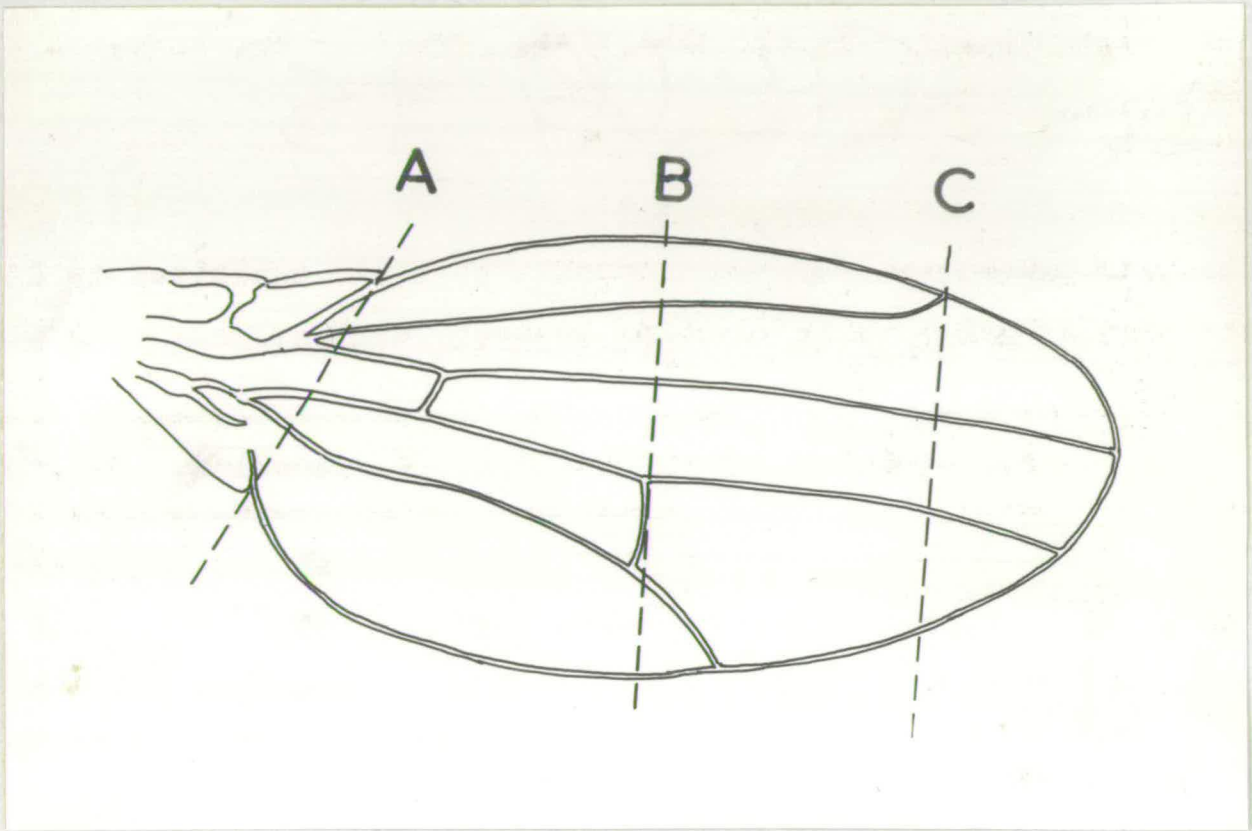
2. Wing area and mating speed, II

I was fortunate to receive from Dr F.W. Robertson two lines which had been selected for high and low ratio of wing length to thorax length respectively. The results of this selection experiment were that the high ratio line (HR) showed an increase of 12% in wing area and the low ratio line (LR) a decrease of 16% while body size remained at approximately the same level as in the controls (Robertson, 1962).

I set up three sets of mass matings using fifty males from each line and from control each time. Control females were used for all the tests in case any change in female receptivity had occurred in the selected lines. Fig. 14 shows the summed results of these experiments and it is obvious that the high ratio males have a faster mating speed than the controls and the low ratio males a lower mating speed.

I also examined samples of single pair matings from each of the lines and control and these results are given in Table 20. The only significant difference is that the low ratio males show more vibration than do the controls ($p < 0.001$). This, however, tends to reinforce the argument as one would expect that this increase in the amount of vibration would compensate, to some extent, the

Fig. 15



Wing of D. melanogaster showing the three positions at which amputations were made.

decrease in wing area. The reason for this increase in vibration was not investigated. It could be due to a random fixation of genes during selection or, it is possible that, as had occurred in the small selected lines, there had been secondary selection for increased vibration to counteract the effect of the decrease in wing area.

Table 20

The mean percentages of the courtship elements exhibited by HR, LR and control males while courting control females.

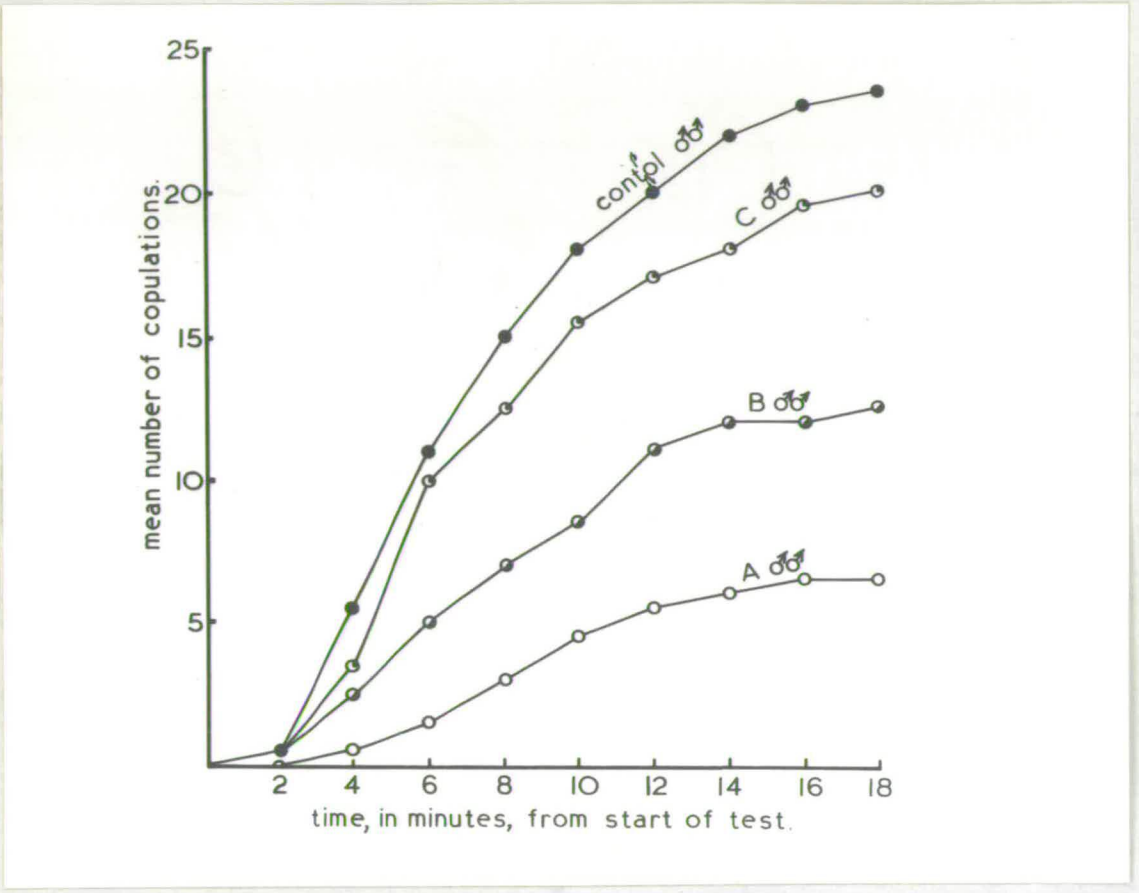
Line	No. of flies	% orientation	% vibration	% licking
HR	9	59	29	12
LR	9	51	39	10
Control	9	66	26	8

Appendix T20.

3. Wing area and mating speed, III

One obvious method of changing wing area is to amputate portions of the wings. This operation is simply done using a scalpel while the flies are etherised. The point where radius (2 + 3) meets radius 1 and the medial cross vein were used as markers thus making it possible to remove, reasonably accurately, standard portions of the wings. Four classes of flies were examined: controls, in which the wings were left intact, and three others with the wings cut at A, B and C as illustrafed in Fig. 15.

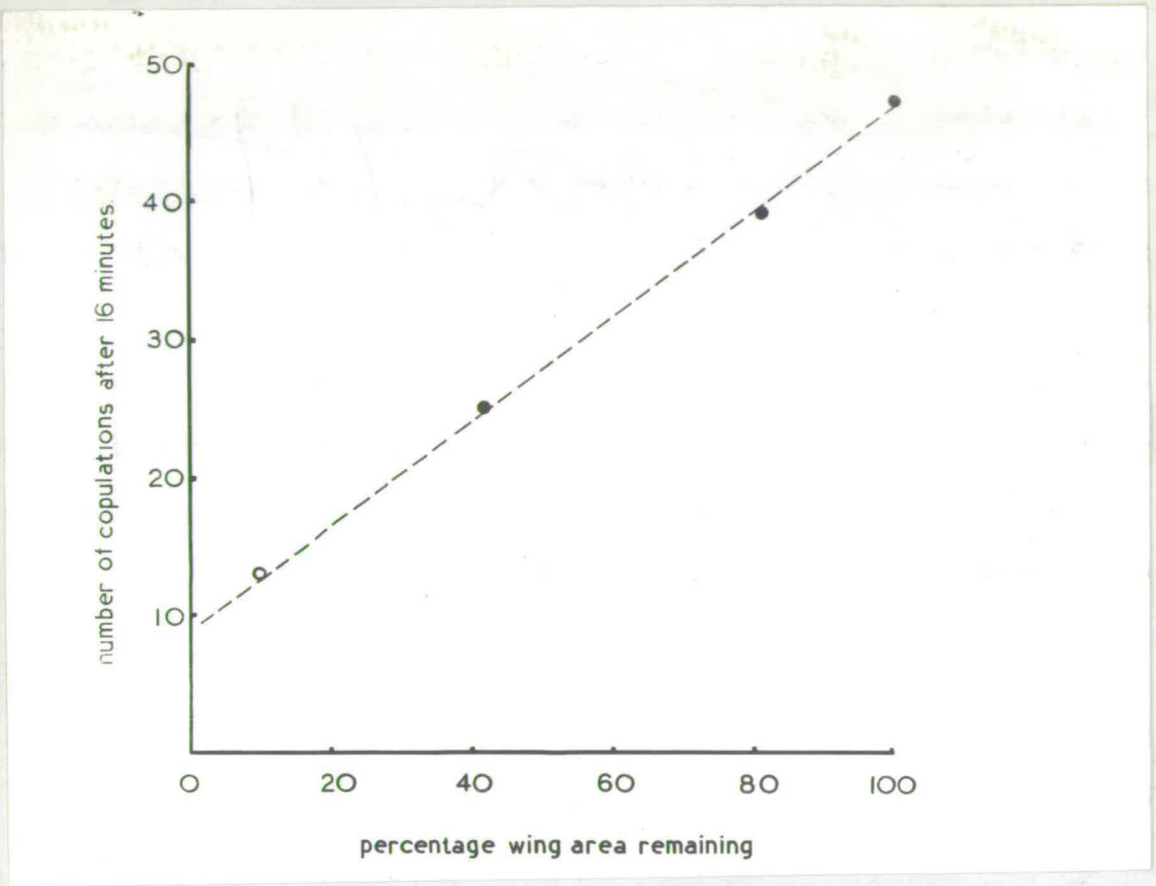
Fig. 16



Mass mating graphs of males with their wings amputated at the different positions illustrated in Fig. 15.

Appendix F16.

Fig. 17



A graph of wing area plotted against number of copulations at 16 minutes and derived from Fig. 16.

Flies with their wings amputated at A were not entirely wingless and it is probable that some sexual stimulation was provided by the wing stumps. It is not, however, easy to entirely remove the wings without damaging the wing muscles in the thorax. The flies treated in this manner seem to court normally. Mayr (1950) and Manning (1959) have already demonstrated that removal of the antennae and fore tarsi respectively do not adversely affect the courtship performance of the males and these operations are likely to be just as serious as amputation of the wings.

Camera lucida drawings of samples of wings from each of the classes were made and their areas calculated as previously described. Table 21 gives the percentage reduction in wing area in the two classes with partially amputated wings.

Table 21

The wing areas of male flies with partially amputated wings expressed as percentages of control wing area.

Control	100	± 0.63%
'C' flies	80.85	± 0.94%
'B' flies	41.57	± 4.79%

Two separate sets of mass matings were set up using fifty pairs of flies from each class on each occasion. The female flies were all normal controls. The summed results of these experiments are shown in Fig. 16 from which it can be seen that mating speed increases with wing area. Fig. 17 shows more clearly the relationship between these two factors. It is a graph of the number of

copulations that occurred during the first sixteen minutes of each test plotted against wing area for the four classes. As it was impossible to measure accurately the area of wing stump left in class A flies I estimated it at 10 per cent. The line joining the points is almost straight and demonstrates clearly the additive nature of the stimulation provided by vibration.

As calculated from the above graph the reduction in mating speed is just over 80 per cent suggesting that this percentage of the total stimulation provided by the males is via vibration.

4. Conclusions

These experiments therefore show conclusively that wing area can have a large effect on mating speed and consequently also on mating success. The heavy selection pressure that must have existed within the small selected lines in order to change the percentage vibration significantly within a few generations is therefore understandable.

CHAPTER VI

Attempts to Change the Levels of Activity by Selection

1. Introduction

The repeatability value for activity as measured in the arena was sufficiently high to suggest that considerable genetic variability for this character existed in the Pacific population. It therefore seemed worthwhile to attempt to change the activity levels by selection. Some of the reasons why this might prove interesting have already been discussed in the introduction. In particular there are several instances of changes in activity associated with changes in courtship behaviour and it is particularly interesting to examine the effects of deliberately modifying activity.

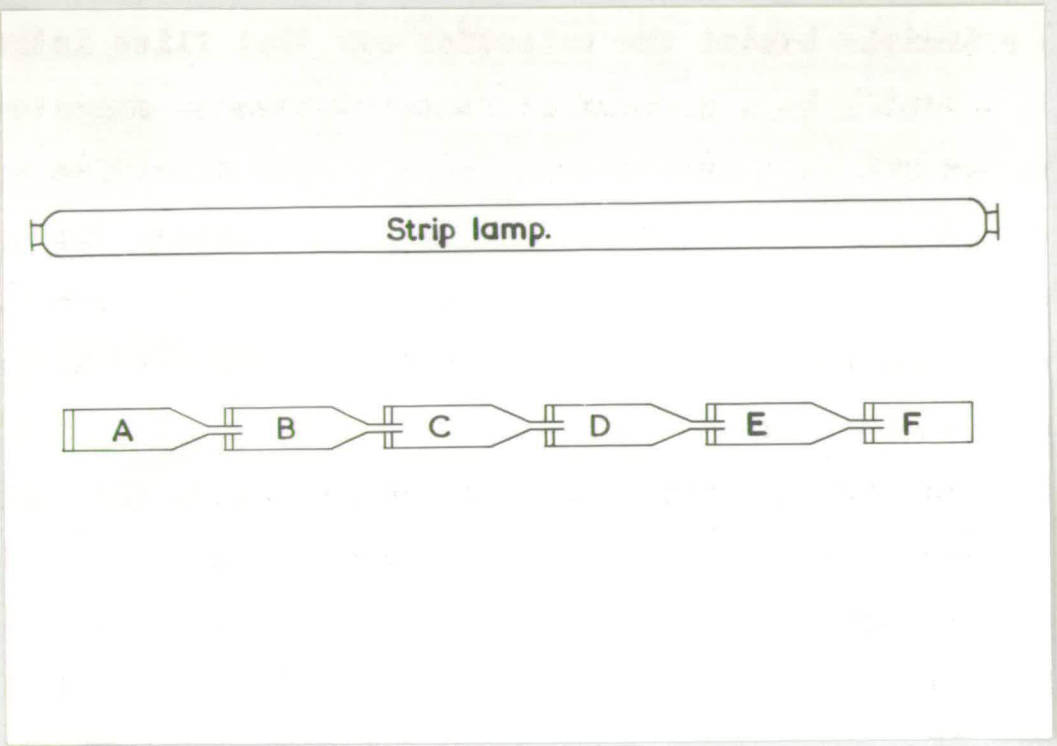
2. Selection for Activity, I

i. Materials and methods

Ideally it would have been best to have selected flies on the basis of their performance in the arena as this is probably the best measure of activity. However, to attempt a two-way selection with two lines selected each for high and low activity would require a minimum of 400 measurements per generation and this is impracticable. I therefore evolved a simpler method of selection.

The methods of selection described here make use of apparatus whose mode of action is similar in principle to that used by Barton Browne and Evans (1960) to measure locomotor activity in relation to feeding and starvation in the blowfly. The apparatus used in

Fig. 18



The apparatus which was used in the first attempt to select for changed levels of activity.

the first experiments is shown in Fig. 18. It consisted of a series of six 2.5" x 1" glass tubes connected by glass funnels having exit diameters of approximately 3 mm. The end pieces of the tubes were of perspex and a 60 watt strip lamp gave an almost uniform light over the entire apparatus.

The principle behind the selection was that flies introduced into tube A would, by a process of random movement, migrate through the tubes towards F. Movement in the opposite direction would be unlikely to occur as the chances of the flies finding the opening in the protruding part of the funnel are small. Observation of the flies in the apparatus showed that in this respect it functioned almost perfectly. Thus the more active flies would arrive first at tube F and the least active would tend to remain in A. On this basis I selected four lines, two for high activity and two for low.

The procedure I used for selecting the flies was as follows. Fifty flies from the Pacific stock were introduced into tube A. These were all virgin, of the same sex and from three to four days old. The first ten to reach F were removed and placed in a milk bottle containing culture medium. I then did the same with the other sex and the ten pairs so selected were allowed to mate and lay eggs for two days after which the flies were discarded. This procedure was replicated so that two active lines (A1 and A2) were initiated. Each succeeding generation I collected fifty males and fifty females from each line and repeated the selection procedure.

The method of selecting for low activity was similar except that the inactive lines (I1 and I2) were initiated and then continued by collecting and breeding from the last ten pairs remaining in A

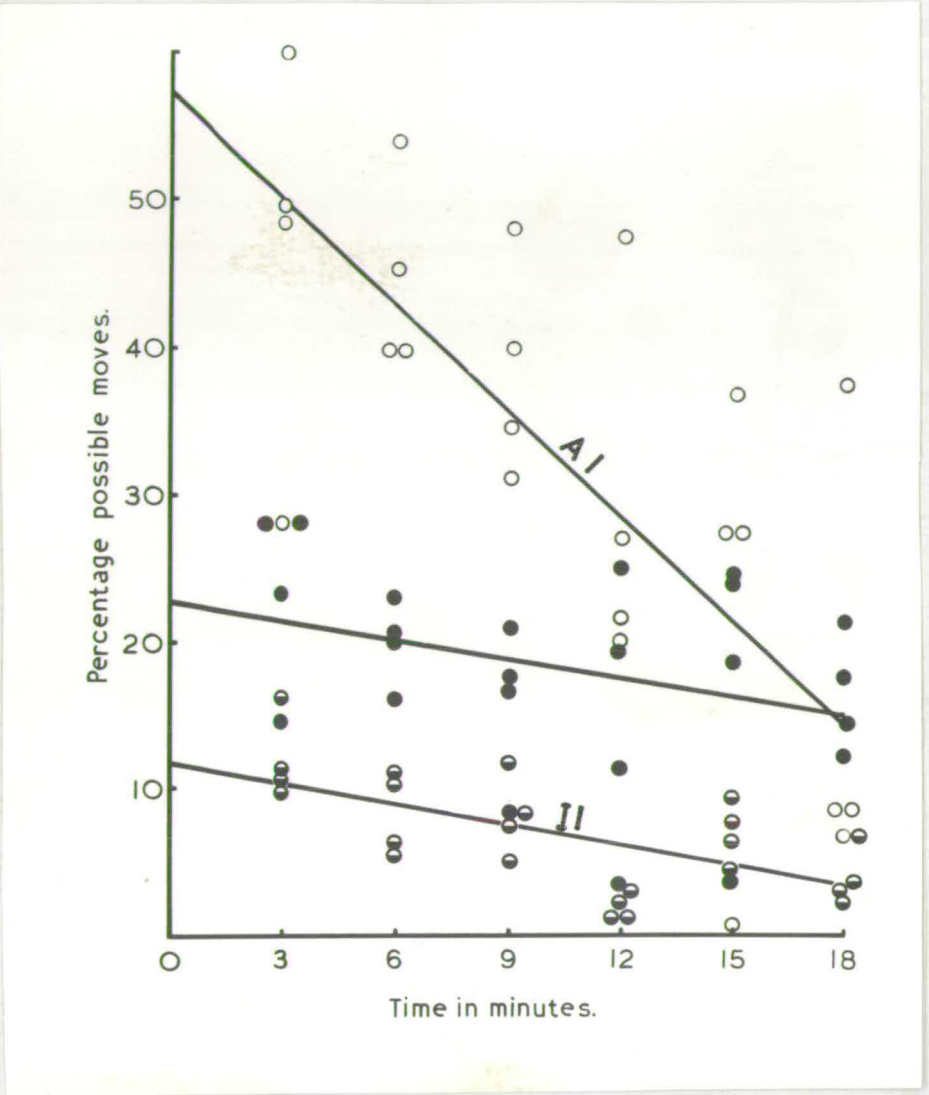
out of the original fifty. A selection pressure of 20% was therefore conferred on the lines each generation. The only departure from the described procedure occurred when, after ten generations, I1 and I2 had responded to selection to such an extent that the majority of the flies did not migrate from the initial tube and it then became necessary to remove ten flies, at random, from those remaining after one hour, and breed from these. The control stock was maintained using ten pairs of unselected flies each generation.

ii. Results

I first tested the success of the selection in the original apparatus. Unfortunately it was not possible to do this each generation and thus obtain a selection response curve but by the eighth generation of selection large differences were apparent between the active and inactive lines. I then examined the lines in the two devices which have previously been used to measure activity, as I thought it would be useful to see how far these methods of measuring activity agreed.

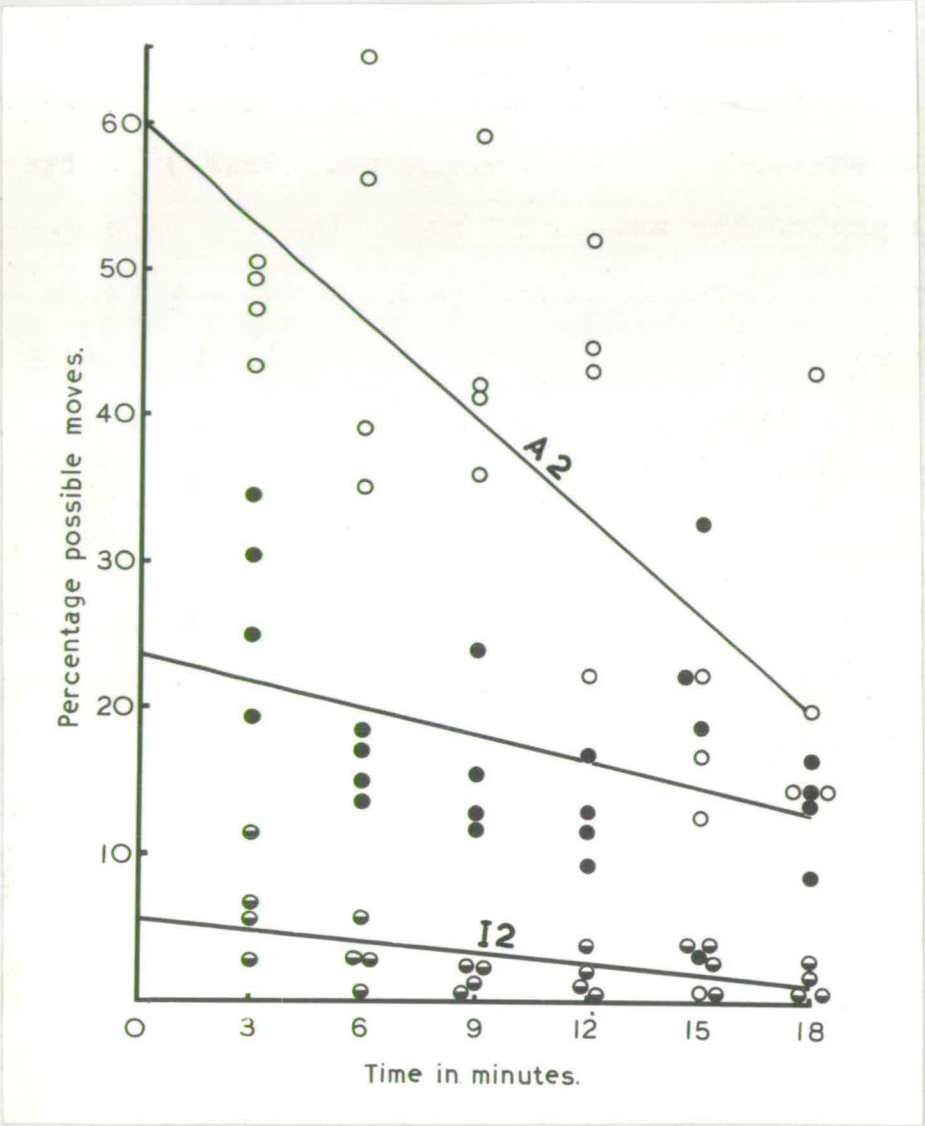
a. I set up, in parallel, three sets of tubes identical to those used during selection. This was done so that one active, one inactive line and control could be examined concurrently. Twenty-five of the same sex were introduced into tube A and, at three minute intervals, the number of flies in each of the six tubes was noted. After eighteen minutes about 80% of the A1 and A2 flies had migrated to the end vial and the experiments were

Fig. 19



Graph showing the activity levels of A1 (O), control (●) and I1 (◒) over an 18 minute period. These are plotted as the percentage possible number of moves from one tube to another in the selection apparatus, within three minute periods.

Fig. 20



Graph as for Fig. 19 showing the activity levels of A2 (○), control (●) and I2, (◒).

therefore terminated at this point. The figures obtained were expressed as 'percentage possible moves' occurring within a three minute period. This was calculated in the following manner. The total number of moves possible are 5 x 25 = 125. (The number of funnels x the number of flies used in each test.) After the first three minute period the number of flies in each tube was noted and thus the number of moves from one tube to another could be calculated. This was represented as a percentage of 125. Similarly the number of moves in the second period can be worked out, however the number of possible moves for this period is 125 minus the number which occurred in the first and the percentage was calculated using this figure. The same method was used for each of the six three minute periods. Two series of experiments were done. In one A1, I1 and control were compared and in the other A2, I2 and control. Each series was replicated so that two samples of males and two of females from each line were tested. I then plotted percentage possible moves against time from the start of the test as shown in Figs. 19 and 20. Regression lines were fitted to the data, the formulae for which are:

A1	$y = -6.66x + 56.12$
Control	$y = -1.27x + 22.54$
I1	$y = -1.39x + 11.64$
A2	$y = -6.69x + 59.68$
Control	$y = -1.85x + 23.67$
I2	$y = -0.76x + 5.46$

These measurements were all done on F12 progeny but samples were also examined on generations 9 and 10 with the same results.

As can be seen there is reasonably good correspondence between A1 and A2, between the two samples of controls and between I1 and I2, both with respect to the slope of the line and, more particularly, the point where the lines cut the y axis. I shall discuss these results later with relation to the results of the other measures of activity but it is worth noting the two important differences between the lines. The first is that at the start of the test the rates of activity differ markedly between the A, I and control lines. Secondly, the level of activity during the eighteen minute period falls off more rapidly in the A lines than in either the I lines or controls.

b. I next examined the lines in the sector apparatus. Twenty-five males and 50 females from each line and control were used. Table 22 shows the results of this measure and these parallel those from the previous experiment. The A lines show higher activity than the controls and the I lines lower activity. Further, selection seems to have been more successful in I2 than I1, as measured by both these methods.

c. Thirdly, I examined activity in the arena of 20 flies, 10 males and 10 females, from each line and control. The results are given in Table 23 and there seems to be little difference between the lines on this criterion. The slightly higher values found in the two A lines do not approach significance.

Table 22

The distance travelled from the centre of the sector of a circle. 75 flies from each line and control in batches of 5.

Line	Number of flies			
A1	0	3	14	58
A2	0	4	7	64
I1	5	17	31	22
I2	21	21	15	18
Control	1	8	21	45
Distance travelled	0	0-2"	2-4"	4-6"

Table 23

The number of squares in the arena entered within 1 minute. 20 flies in each group.

Line	A1	A2	I1	I2	Control
Mean squares entered	76.2	82.1	69.3	62.7	69.4
Range	35-116	28-114	13-106	27-90	30-102
s	17.20	20.55	21.90	14.45	17.25

iii. Nature of the Selection Response

There is one obvious possibility as to why the first two methods of measuring activity gave differences between the lines and the third did not. In the former activity was measured using

groups of flies and in the latter single flies were examined. The possible drawback of the sector method, in that it does not eliminate the possibility of interaction between flies, has already been mentioned. Selection could have influenced the 'reactivity' of flies to one another by increasing and decreasing avoidance reactions. This type of difference has been shown to exist between natural populations of D. melanogaster by Saki et al. (1958).

To see whether there was any observable difference between lines in the way in which the flies reacted to one another I introduced pairs of females in the perspex cells used for examining single pair matings and watched the flies under a low power binocular microscope. I used female rather than male flies as their reactions to one another would not be influenced by courtship responses. I noticed an immediate and obvious difference between the behaviour of flies from the A and I lines. When females from A1 and A2 were introduced into the cells they ran around and met frequently. These meetings were accompanied by kicking, wing flicking and jumping which are all repelling movements (Bastock and Manning, 1955). After about 15 seconds the flies settled down to circling the wall of the cell about 180° apart and further encounters between them were rare, the flies maintaining as great as possible a 'spacing distance' between one another. I1 and I2 flies, on the other hand, ran around far less vigorously and meetings between them very seldom resulted in any obvious repelling movements. They did not show the circling pattern of the A line flies and moved around the cells seemingly at random. These observations reinforce the hypothesis that selection has changed, not the levels of spontaneous activity, but

the flies' reactivity to one another.

The differences between the A lines and controls in Figs. 19 and 20 can probably now be explained satisfactorily. The initial high rate of activity in the A lines would be due to their greater reactivity. However as the flies migrated along the tubes so their density per tube would decrease. Further, the most reactive flies would reach the end tube first and would thus be progressively eliminated from the nearer tubes. These two factors could account for the rapid drop in activity to control level exhibited by the A lines during the course of the experiment. For these reasons the points at which the regression lines cut the y axis and not the slopes of the lines must be taken to obtain a comparative measure of reactivity.

iv. Courtship behaviour

It seemed probable that the changes in behaviour of the type obtained by these selection experiments, affecting as they do the interactions between flies, would also be likely to affect courtship and I therefore examined this aspect of their behaviour.

As the A and I lines were replicated I examined the courtship behaviour of one of each only. I recorded the courtships of males from each line courting both their own females and those from the other line. The courtship of controls was also recorded. By comparing the results of these two types of courtship some idea of the relative importance of male and female roles can be obtained. I analysed these courtships more fully than usual and calculated, along with the percentages of the courtship elements, the number

of breaks in courtship and their duration. The results obtained are summarised in Table 24.

The courtship of A2 males with their own females contains a lower proportion of high threshold elements than either controls or I2 males with their females. Also, they have more and longer breaks in their courtships. This result is what would be expected if the high reactivity of the A2 flies was competing with their sex drive. Similarly the I2 flies, whose reactivity is low, exhibit higher intensity courtship than controls, i.e. their courtships contain a greater percentage of the high threshold courtship elements and the breaks in courtship are fewer and shorter.

The influence of the females can be clearly seen from the results of the inter-line courtships. I2 males when courting A2 females exhibit behaviour similar to that of A2 males with low intensity courtship. The high reactivity of the A2 females obviously has a strong inhibitory effect on male courtship. Conversely, A2 males courting I2 females show more intense courtship than with their own females but the results suggest that their own high reactivity also has an effect in that their courtship in this situation does not reach the same level of intensity as that of I2 males with I2 females or of the controls. These results are comparable to those of Bastock (1956) who showed that males courting stationary females showed a higher intensity of courtship than when courting females that were moving.

Table 24

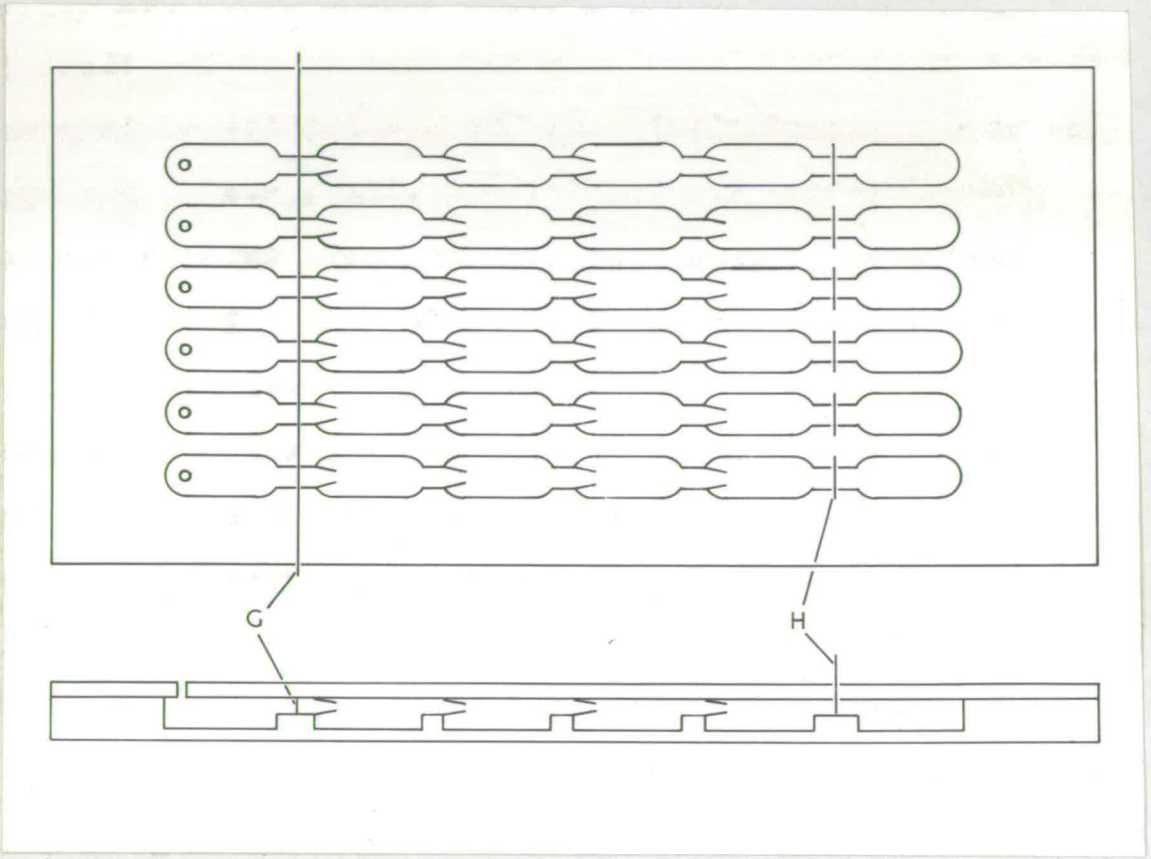
Summary of courtship behaviour of A2 and I2 males

Courtship type		Number of courtships examined	Average percent orientation	Average percent vibration	Average percent licking	Average number of breaks	Average length of breaks *
Control	Control	10	54.1	34.5	11.4	1.0	5.55
I2	I2	20	46.6	40.3	13.1	0.45	2.78
A2	A2	20	68.7	24.5	6.8	1.8	6.15
A2	I2	15	59.0	29.0	12.0	0.27	2.33
I2	A2	15	68.4	25.6	6.0	2.0	9.50

Appendix T24.

* In metronome units of 1.5 seconds.

Fig. 21



The apparatus which was used in the second attempt to select for changed levels of activity.

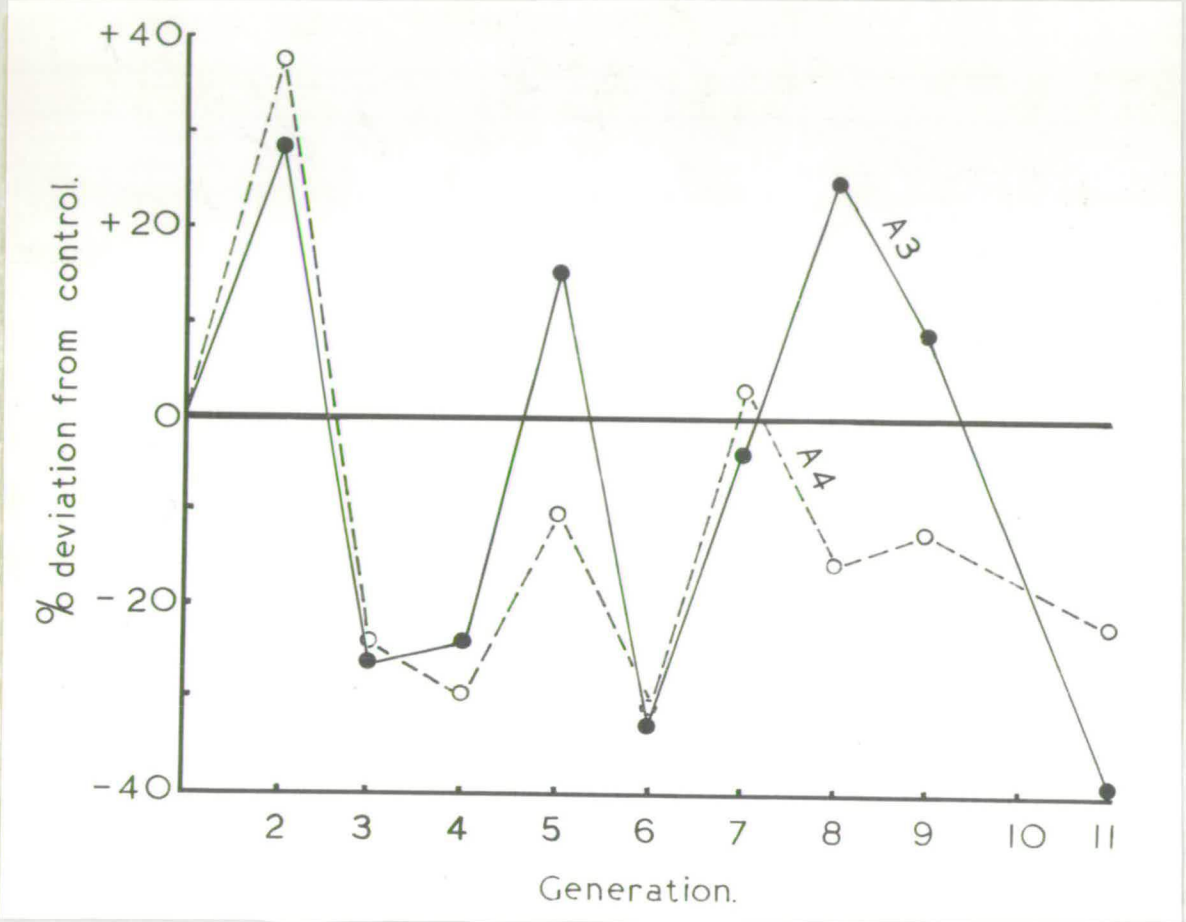
3. Selection for Activity, II

i. Materials and methods

As the previous selections did not succeed in changing the levels of spontaneous activity I tried a second selection experiment. Its form was suggested directly by the results of the first in that the flies were measured singly and the possibility of interactions between flies affecting the results was eliminated. The apparatus used is illustrated in Fig. 21. In principle this is the same as the previous one and consists of cells 32 x 13 x 10 mm. connected by funnels with 3 mm. exit diameters. There were six cells in each line, and thirty lines, in banks of six, were constructed. The apparatus was carved from a block of perspex and the funnels made from a clear plastic. A transparent perspex lid covered the cells but the sides were opaque so that flies in each line of cells were visually isolated from one another. A gate, G, closed off the first cells from the rest and a series of further gates, H, could be lowered to trap flies as they reached the last cells.

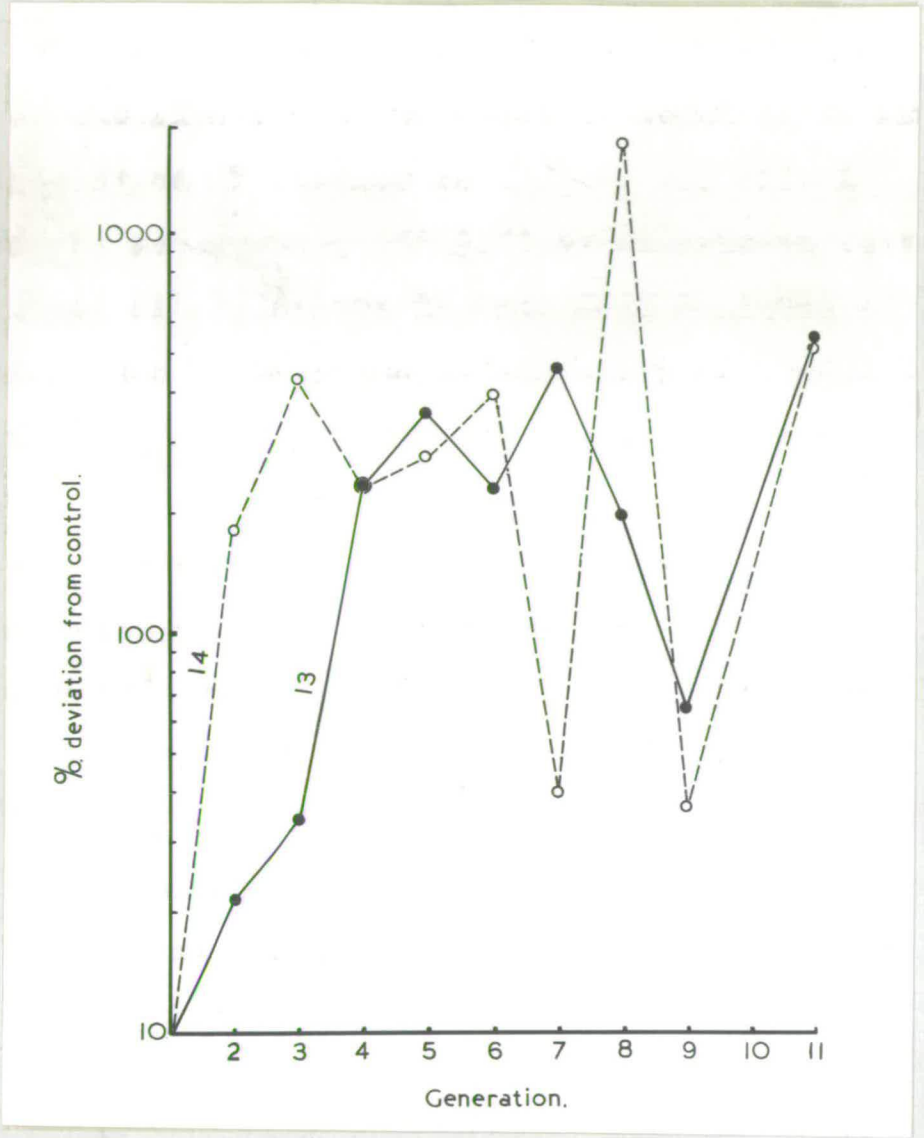
I selected two lines for high activity, A3 and A4, and two for low, I3 and I4, and the procedure was the same in all respects as in the previous experiment, save the following: one fly only was introduced into a line of cells and thus 30 flies at a time could be run through the apparatus, their release from the first cell being synchronised by use of the gate. 60 flies per line per sex were used each generation, the 12 most active and the 12 least active in each line respectively being used for breeding. The criteria for activity were the same as previously, the least active being the last ones remaining in the first cells and the most active the ones that reached the final cells first.

Fig. 22



The selection response of A3 and A4. Successful selection would have resulted in the lines taking less time than controls to pass through the selection apparatus. This would be shown on the graph as minus values. The response in the selected direction in generation 11 is no greater than are the fluctuations, in the opposite direction, in generation 2.

Fig. 23



The selection response of 13 and 14 expressed as percentage deviation from control. There was no selection on generation 10.

ii. Results

The time taken, in the case of the A3 and A4 lines, for the 12 flies of each sex to reach the end cell, and in that of I3 and I4 for 48 out of 60 flies to leave the first cell was noted each generation. I also ran samples of control flies through the apparatus every generation so that the performance of the selected lines could be compared with that of control. As the apparatus held only 30 flies, 20 runs, including those of controls, had to be carried out each generation. Introducing flies into the apparatus and subsequently removing the selected ones took an appreciable length of time and therefore the selection experiments were spread over two and sometimes three mornings. Thus, between day variability was added to the within day variability of the measures and the selection response seems rather erratic. Figs. 22 and 23 show the responses of the A and I lines over 10 generations of selection. (There was no selection on generation 10.) These figures are plotted as percentage deviation from control as this eliminates the fluctuations that occur between generations that are common to all the lines and to control. As can be seen from Fig. 23, I3 and I4 show an immediate response which, after generation 4, flattens to a level where the two I lines are taking approximately from 11 to 14 times as long as control for all but 12 flies to vacate the initial cells. The immediacy of this response suggests that relatively few genes are concerned in the change.

The results of selection for increased activity as shown in Fig. 22 indicate that selection has not succeeded. The degree of

deviation on generation 11 is no greater in the direction of selection than is the presumably random fluctuation in the opposite direction during generation 2.

iii. Nature of selection response

I carried out the following tests on generations 12 and 13 of selection to determine the nature of the response obtained. Thirty flies from each line and control were examined in the arena. Table 25 shows the results of these measures. I also examined the A3 and A4 lines so as to eliminate the possibility that selection had had some small effect that was masked by the considerable variability of the measures obtained in the selection apparatus. As can be seen there seems to be no significant difference between any of the lines and control with regard to this measure. Once again the arena test failed to agree with the experimental one in its measure of activity.

Table 25

The number of squares in the arena entered within 1 minute.
30 flies in each group.

Line	A3	A4	I3	I4	Control
Mean squares entered	29.4	34.2	25.3	30.6	28.3
Range	8-59	10-64	4-55	6-57	12-63
s	12.7	17.0	14.6	17.0	16.5

Appendix T25.

There was one obvious difference between the stimulus situations presented to flies in the selection apparatus and in the arena. In

the former the flies had to pass through a series of narrow funnels and the difference between the I lines and control could be due to the way in which the flies reacted to these funnels. The following experiments were therefore done in order to explore this possibility.

One fly at a time was introduced into the initial cell of one of a series and the gate removed. The fly was watched for 5 minutes or until such time as it moved to the second cell. Each time that the fly entered the funnel was noted as was the time that it finally left the cell. Fifteen male flies from each of the I lines and from control were examined. Of the control flies 8 passed through the funnel immediately they entered it, 6 went through on the second 'attempt' and one on the fifth, giving an average of 1.61 attempts per fly. Averages were worked out in the same way for the I3 and I4 flies and were 5.87 and 5.09 respectively, few of these flies going through in the first two attempts. A figure was also calculated by dividing the average length of time spent in the cell by the average number of times the flies entered the funnel. As the flies would be expected to find the funnel at random this figure will give an idea of the level of spontaneous activity. Controls took an average of 11.93 seconds per attempt, I3, 14.13 seconds and I4, 13.30 seconds. These differences between the lines and control are not significant. These results therefore show that the differences found were due, not to changed levels of activity, but to the willingness of the flies to go through the narrow funnels. They also explain why selection for increased activity was a failure in this apparatus. There is obviously not much room for improvement in performance of the control flies as, except for one individual,

all went through the funnel on either the first or second attempt. I examined the following three possibilities to account for the change in the I3 and I4 lines.

a. Selection had changed body size and the I line flies were larger than controls. They would therefore not be able to pass through the funnels without touching the walls.

b. As the funnels were made from a plastic which differed in composition from the perspex of the rest of the apparatus, the I3 and I4 flies could be reacting differently either to the quality of the light transmitted through the funnels or to some chemical components in the plastic.

c. Selection may have been for some 'claustrophobic' effect due either to visual or tactile stimuli from the walls.

I tested the first possibility by measuring the thorax lengths of 10 male flies from each of the I lines and control. The thorax lengths were as follows:

A3	0.992 ± .016 mm.
A4	0.984 ± .0368 mm.
Controls	0.976 ± .0268 mm.

There is obviously no significant difference between the lines and control in body size.

I used the arena to test the second possibility. Half the internal surface of the arena was covered with the same plastic as was used to make the funnels. Ten I3 males and 10 control males were used. They were introduced singly into the apparatus in the usual way and watched for 5 minutes each. The lengths of time that they spent in the two regions of the arena were measured. I3 males

spent 33.7% of the time in the plastic lined areas and the controls, 34.3%. It is interesting that less time was spent by both samples of flies in the plastic lined portions of the arena but the reason for this was not investigated. It could be due to the decreased amount of light in these areas. However there is obviously no difference between the two samples with regard to this measure, thus eliminating possibility b.

To test the third possibility, I constructed a hollow wedge from perspex. This was 6 inches long, marked off at 1 inch intervals 1 cm. broad and tapered in height from 6 mm. to zero along its length. A fly was introduced through an aperture in the high end and its position along the length of the apparatus noted every second for a period of 50 seconds. Thus the percentage of time spent in each of the inch long portions of the apparatus could be calculated.

I examined 20 males from I3 and from control. Table 26 shows the results of this measure, the height of the apparatus decreasing from 1 to 6. As can be seen, the I3 flies spent more time in portions 1 and 2 than controls and progressively less time in the remaining four narrower portions. The number of flies that spent from 0 - 10%, 11 - 20%, 21 - 30%, 31 - 40% and over 40% of the time in each of the six regions of the apparatus was worked out. This gave 30 categories but, as certain of these contained few or no figures, the data was condensed to give at least five units per category in the controls. A χ^2 was then calculated using controls as the expected values. The results were highly significant.

$$\chi^2 = 39.20$$

$$d.f. = 10$$

$$p. = < .001$$

Table 26

The average percentage time spent by 20 flies from both I3 and Control in the six regions of the wedge. The height of the wedge decreases from 1 to 6.

Line	1	2	3	4	5	6
I3	29.6	33.3	24.2	11.4	1.5	0
Control	26.7	22.3	24.8	19.3	6.6	0.3

One further experiment was carried out to see whether this reluctance on the part of the I3 flies to enter the narrower segments was due to visual or tactile stimuli. The former was more likely as a fly walking on the floor of the wedge would not touch the roof until about one third of the way down the fifth segment. The experiment was repeated and extended to include the I4 line, 10 males from each line and control being used. Also, a further 10 males from each were examined in the apparatus, the source of illumination being shielded by a red filter (Kodak Wratten filter, No. 2). It is unlikely that the flies can see in this light (Bastock, 1955).

Table 27 shows the results of these experiments. Under normal illumination both I3 and I4 spend more time in sector 1 than control about the same in 2 and a progressively smaller percentage of time in each of the successively narrower portions of the wedge, thus confirming the results of the previous experiment. A similar statistical test to that already described was employed, the figures from I3 and I4 being lumped as the samples were, in this experiment, unavoidably smaller than in the previous one. The results were

significant at the 5% level:

$$\chi^2 = 33.05$$

$$d.f. = 17$$

Under red light flies from the two selected lines do not seem to spend more time in the wider portions of the wedge than do the controls and a ^{2} calculated from these results was 19.94 which, with 17 degrees of freedom, gives a probability of between 0.30 and 0.20. It would therefore seem that the stimuli preventing the I3 and I4 flies from passing through the funnels in the selection apparatus were visual.

Table 27

A comparison of the average percentage time spent by 10 flies from each of I3, I4 and control in the six regions of the wedge under conditions of normal illumination and red light.

Normal Illumination

Line	1	2	3	4	5	6
I3	40.6	20.8	17.4	14.6	6.4	0.2
I4	38.0	26.2	16.2	14.0	5.6	0
Control	25.6	21.2	21.6	19.8	11.2	0.6

Red Light

Line	1	2	3	4	5	6
I3	34.6	20.4	22.0	17.4	5.6	0
I4	23.8	21.2	23.0	17.8	14.2	0
Control	35.0	19.6	16.6	16.6	11.6	0.6

4. Discussion

Neither of the two selection experiments fulfilled the original intention of changing the levels of activity, at least as measured by the arena. This is surprising in view of the high repeatability of activity measured in the arena and also as both Manning (1961) and I have shown that large differences do exist between lines of flies tested in this way and these differences must be due to changes in genotype. Activity, however, must reflect many aspects of the animals' behaviour such as the relative strengths of sex, hunger and exploratory drives along with factors such as 'tameness'. In both these experiments selection must have acted on the most 'accessible' characters which were not necessarily the most obvious ones. The highly specific natures of the responses are also interesting; in each case only one aspect of behaviour seemed to have been directly affected.

The response obtained in I3 and I4 was somewhat bizarre and it is not obvious why variability for this 'claustrophobic' character exists in the population. Possibly this is a side effect of some more adaptive character which was not revealed by the methods of measurement used. Also, there is the possibility that, in the wild, there is a strong selective advantage for flies that avoid small spaces where they could get smothered in the food. This situation does not normally arise under laboratory conditions and this trait might have become lost during the process of domestication.

The changes in reactivity that were obtained in the first selection experiments were not altogether unexpected. This is a character with an obvious adaptive significance. Too high a level

of reactivity would affect courtship adversely as already shown and might tend to space the flies out more than was ecologically advantageous. Too low a level of reactivity could lead to over-crowding and inhibit flies from migrating and thus finding new food sources and mates (Sexton and Stalker, 1961).

These results also emphasise the necessity of describing very carefully the apparatus used in selection and the conditions under which activity is measured. It is also important to examine individual animals in the test situation to see, if possible, which stimuli in the environment they are reacting to as there is little value in obtaining a selection response whose character remains unexplained.

GENERAL CONCLUSIONS

The experiments described in this thesis cover a somewhat diverse field of behaviour and it has been considered convenient to discuss specific points as they arose in the text. There are however a few general conclusions that can be added here.

It was stated in the introduction that studies on the genetics of behaviour should tell us both about the possible directions of evolutionary change and something about the organisation of behaviour. It is necessary to consider how far these aims have been realised. The high measures of repeatability for activity and courtship intensity indicate that there is probably considerable genotypic variability for both these characters in the population that I examined. The lines which had been selected for small body size, in which the courtship intensity had changed due to secondary selection, demonstrate that this genotypic variability for courtship behaviour enables adaptive changes to occur. This provides a model to illustrate the way in which courtship could change under natural conditions. The differences that are found between closely related species are usually of this type, that is they are quantitative and not qualitative, as shown for example by Manning (1959a, 1959b) in comparative studies of D. melanogaster and D. simulans. One way in which changes in courtship intensity could possibly lead to sexual isolation has been discussed by Bastock (1955, 1956). Lowered courtship intensity, that is, a lower proportion of vibration in male courtship due to the pleiotropic action of otherwise advantageous genes, could confer a selective advantage on females

which responded more to the visual aspect of sexual stimulation and thus the pattern of courtship behaviour could gradually change.

Changes in wing area and reactivity directly affected mating success and could therefore provide the stimulus for changed courtship behaviour. In every case selection, whether for morphological or behavioural characters, changed not only the character under selection but also affected some other aspects of the flies' behaviour. The pleiotropic action of genes is, of course, general and these examples demonstrate the usefulness of extending the investigation of pleiotropy to behavioural characters.

The various ways in which activity and courtship behaviour were found to interact by Manning (1961) and myself indicate that, in melanogaster, it is not possible to uphold a concept of 'vigour' which would embrace many aspects of behaviour. Each component of behaviour will be individually maintained at a characteristic level due to the selection pressures acting directly on it and also due to its interaction with other characters behavioural and otherwise. Thus a character will be kept at a compromise and not at a maximum level. For example, there may be selection pressure for increased wing area due to the advantage that this would confer on courtship. As wing area also affects flight for which very large wings will be disadvantageous, a counteracting selection pressure will, at the same time, tend to decrease wing area. It is therefore necessary to examin as wide a range of behaviour as is possible when one selects for or otherwise changes a behaviour pattern in order to gain a proper appreciation of the way in which behaviour is integrated. Some of the possible interactions that can exist between body size, wing

area, courtship, reactivity and activity are described in this thesis.

Of all the genetical techniques that were used, the selection experiments provided the most interesting results. The ease with which responses were obtained through selection emphasises both the usefulness of the method and the need to use carefully controlled criteria for selection. There is little advantage in selecting lines the differences between which can only be demonstrated in the selection apparatus itself and whose relevance to possible evolutionary trends is unexplained. Unfortunately it is not always possible to predict the outcome of selection experiments and occasional anomalous results must be expected.

The application of certain of the quantitative techniques to behavioural characters was not successful, particularly the attempt to separate the environmental and genotypic components of variance. This experiment should be repeated and extended to include crosses between other inbred lines. If wide differences in variance estimates were found between such crosses then it would be necessary to re-examine the theoretical basis of this method of separating VE and VG as applied to behavioural characters. A fuller genetical analysis using backcrosses and analyses of the F2 progeny could also provide some information.

Although a working definition of activity was given in the introduction no very clear idea of what this term entails has arisen from the experiments that I have carried out. One gains a subjective impression, which is probably valid, that different species have characteristic basal activity levels. However,

superimposed upon basal activity will be the effects of both the environmental stimuli and the motivational state of the animal. Thus it is experimentally impossible to arrive at a measure of basal activity as the environmental stimuli can never be entirely removed nor can one ever say that an animal is without specific motivation. The safest use of activity is therefore probably in conjunction with other factors. For example activity levels during food deprivation or under differing environmental conditions can tell us something about the adaptiveness of behaviour. However, as I have used the arena as a standard measure of activity it would still be interesting although laborious to select for changes in activity as measured by this method and to attempt to analyse the factors responsible for such changes.

I failed to discover the cause of the fluctuations in behaviour that were found between days and generations and it would be instructive to rear the larvae and also to feed the adults on sterile, defined media to see whether these fluctuations could be eliminated. The evidence that I obtained, which was mainly negative, did suggest that some factor in the food was implicated and while it would probably be difficult and tedious to discover the exact cause of the fluctuations, such an experiment would narrow the field greatly.

Finally, it would be instructive to compare the behaviour of truly wild strains with that of the domesticated stock that I used. While there is no doubt that selection pressures are acting all the time on the laboratory stocks, these are bound to differ from the selection pressures found under natural conditions. It would be

interesting to know at what levels courtship intensity and reactivity, for example, are normally maintained.

SUMMARY

This thesis is concerned with two main aspects of the behaviour of Drosophila melanogaster: courtship of the males and general activity. The following is a summary of the main points that emerged.

1. There is considerable variability in both these measures which can for convenience be split into within day, between day and between generation components.

2. The within day variability can, in part, be attributed to a diurnal rhythm which probably affects all aspects of behaviour and the mating speed had peaks at 'dawn' and at 1930 hrs. ('dusk' was at 2330 hrs.).

3. I calculated the repeatability for measures of courtship behaviour and activity. In both cases the values were in the region of 0.8 and this indicates:

   a. The measures employed were behaviourally meaningful.

   b. The experimental methods were accurate.

   c. There probably exists considerable genotypic variability for both characters.

   d. Female behaviour, during courtship, as long as outbred virgin females of the same age were used, did not greatly contribute to the variability of male courtship behaviour.

4. An attempt was made to separate the genotypic and environmental components of variance for activity and courtship. The results obtained were absurd and a possible flaw in the method was discussed.

5. Much of the variability observed in the behavioural measures

must be due to some environmental factor or factors and therefore the following experiments were carried out in an attempt to discover the cause of this variability.

a. Lowering the temperature at which the tests were carried out by 10°C reduced both activity and mating speed. The reduction in activity was more consistent with the hypothesis that the temperature was acting on the nervous system rather than on the general metabolism of the flies.

b. Lowering the temperature by 8°C during development of the larvae and pupae resulted in flies that had large wings. Such male flies exhibited a faster mating speed than the controls while their courtship intensity remained the same.

c. Reduction in light intensity had no detectable effect on either courtship or activity.

d. Rearing the larvae in sub-optimal conditions of nutrition produced flies of small size. The males exhibited less intense courtship than controls.

6. Examples were given where the fluctuations in behaviour between days was greater than that caused by any of the experimental changes in the environment described above. The cause of the variability therefore remains obscure. However, from an experimental point of view this variability is not too important as experiments can be designed to allow for it.

7. Selection for a morphological character can often have large secondary effects on behaviour. In order to test for this I obtained six lines of flies three of which had been selected for large body size and three for small. The males from the large

selected lines exhibited lower intensity courtship than controls while, conversely, the small selected males exhibited higher intensity courtship. I examined the following possibilities to explain the divergence that had occurred in courtship behaviour.

a. There is a straightforward phenotypic connection between body size and courtship intensity. This was unlikely as reducing body size by crowding the larvae resulted in lowered courtship intensity. I also examined the courtship of a sample of control flies and subsequently measured the body sizes of the males. No correlation was found between body size and courtship intensity.

b. The genes controlling body size (or genes closely linked to them) could have a pleiotropic effect on courtship. To test this I examined the courtship of the F2 progeny of a cross between the large and small lines. The body sizes of these males were also measured and once again there was no correlation between body size and courtship.

c. The divergence in courtship could be due to changed levels of physiological efficiency or metabolic rate. This could be reflected in changed activity levels. However, all the selected lines were less active than controls and thus activity did not parallel the courtship divergence.

d. There could have been a secondary selection pressure acting on courtship behaviour. I therefore compared the sexual success of males from the selected lines with that of control males. Small selected males, in spite of their increased courtship intensity, were less successful than controls.

Large selected males were equally successful as control males although their courtship was of lower intensity.

8. As one would expect that high courtship intensity would be rewarded by a high level of sexual success these results suggested that there could have been selection for high intensity courtship in the small selected lines to counteract some deficiency in their courtship. I therefore tested this in the following manner.

a. I selected lines of flies for large and small body size in such a way that sexual competition between males was eliminated.

b. The sexual behaviour of these small selected males did not diverge from that of the controls over nine generations of selection.

c. The courtship behaviour of the large selected line diverged in the same manner as in the original lines. As there was no difference between the controls and the large males with respect to sexual success it was unlikely that the divergence of their courtship was due to some secondary selection pressure. Therefore the persistence of this divergence in the absence of sexual competition was to be expected.

d. On reintroducing sexual competition in the small selected line and continuing selection for small body size I found that the courtship behaviour of these males once again diverged in the same manner as in the original small selected lines.

e. This experiment demonstrated that the increase in courtship intensity in the small males was due to a secondary selection pressure acting on courtship and tending to compensate for some deficiency in courtship which had resulted from selection for

small body size.

f. The causes of the large selected males' reduction in courtship intensity was not investigated further.

9. The hypothesis that I suggested to reconcile the differences in courtship intensity and sexual success was as follows. The small selected males, partly due to their smaller wings, provided less sexual stimulation via vibration than controls and were therefore less successful in obtaining mates. Conversely the large selected males with larger wings provided more stimulation than the controls and thus, in spite of their lower intensity courtship behaviour, are equally successful, them. I carried out the following experiments to test whether this hypothesis was tenable.

a. Evidence of males with large wings having an increased sexual success has already been given (5b).

b. Two lines which had been selected for large and small wing area respectively were obtained. Males with large wings had faster and those with small wings slower mating speeds than had controls. These differences in mating speed (and consequently mating success) could not be due to differences in courtship intensity.

c. Finally I carried out mass matings using samples of males that had portions of their wings amputated. They showed that there was a linear relationship between mating speed and wing area. Extrapolating from these results it appeared that at least 80 per cent of the sexual stimulation was normally due to vibration. It is therefore quite possible that differences in wing area and courtship intensity could be of considerable selective importance.

10. To examine the genetic basis of activity and to try and discover more exactly what this term entailed I attempted to select lines for high and low levels of activity with the following results.

a. As measured in the selection apparatus there was a large selection response in both directions of selection.

b. Tests of activity carried out on individual flies failed to show any difference between the lines.

c. However, tests in which five flies at a time were examined gave similar results to those in the selection apparatus.

d. As a mass selection technique had been used where the flies had not been isolated from one another it seemed possible that selection had changed, not the levels of activity, but of re-activity to one another.

e. I therefore examined the behaviour of pairs of female flies in small cells. Females from the two 'reactive' lines repelled one another and kept as large as possible a spacing distance between them. On the other hand those from the 'unreactive' lines did not behave in this way as they frequently met and did not make repelling movements.

11. As these behavioural differences affected the interactions between flies it seemed probable that they would have some effect on courtship and I therefore examined this aspect of their behaviour.

a. The courtship of reactive males with their own females was of a lower intensity and contained more and longer breaks than that of unreactive males with their females. This is what one would expect if, in the former, the flies' increased reactivity was interfering with courtship and in the latter decreased

reactivity was facilitating courtship. Controls were intermediate between the two selected lines.

b. I also examined reactive males courting unreactive females and unreactive males with reactive females. In the former case the reactive males showed a higher intensity courtship than when paired with their own females. The courtship intensity of the unreactive males was decreased when they were paired with reactive females.

12. As the previous experiment had failed in so far as the 'spontaneous' activity levels were not changed I attempted a second selection in which any possible interactions between the flies during selection were eliminated.

a. No selection response was obtained for increased activity as measured in the selection apparatus.

b. Selection for decreased activity as measured in the selection apparatus did succeed.

c. The decrease in 'activity' was not apparent when other methods of measuring activity were used.

d. I therefore examined closely the behaviour of the flies in the selection apparatus. The criterion for selection was the speed with which the flies travelled through a series of chambers by way of 'one-way' funnels. Control flies passed through these funnels extremely quickly while the 'inactive' flies refused to do so although the amount of running that both types of fly did in the chambers was the same. It therefore seemed that once again the character that had been selected was not, in fact, activity.

e. The speed at which the controls passed through the apparatus was probably near an optimum thus explaining the failure of selection in the direction of increased 'activity'.

f. I examined the reactions of these 'claustrophobic' flies further and demonstrated that their reluctance to pass through the funnels was due to the reception of visual stimuli from the walls of the funnels as the difference between the controls and selected flies disappeared in the dark.

13. The extent to which these results contribute to an understanding of the organisation of behaviour and indicate possible directions of evolutionary change are discussed along with suggestions for further research.

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Publications

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Appendix T1.

Activity in the arena. Figures on which the calculation of repeatability are based.

Sample 1. Females.

No.	Test		
	A	B	C
1	48	81	43
2	47	64	60
3	56	69	63
4	48	38	46
5	19	15	14
6	31	1	49
7	34	72	44
8	49	52	17
9	38	41	44
10	42	44	61

Source of variation	Sums of squares	Degrees of freedom	Variance estimate
Between flies	5577	9	619.7
Within flies	4106	20	205.3
Total	9683	29	

Sample 2. Males.

No.	Test		
	A	B	C
1	37	29	51
2	75	78	65
3	63	69	45
4	52	7	39
5	112	99	87
6	62	38	66
7	86	63	78
8	53	57	78
9	46	68	63
10	87	86	72

Source of variation	Sums of squares	Degrees of freedom	Variance estimate
Between flies	10513	9	1168
Within flies	3537	20	176.9
Total	14050	29	

Appendix T2.

Percentage orientation. Figures on which the calculation of repeatability are based.

Sample 1

No.	Test		
	A	B	C
1	52	41	45
2	41	42	43
3	55	50	48

Source of variation	Sums of squares	Degrees of freedom	Variance estimate
Between males	122	2	61
Within males	90	6	15
Total	212	8	

Sample 2

No.	Test		
	A	B	C
1	71	53	70
2	68	67	70
3	54	45	44
4	51	50	60
5	62	58	35

Source of variation	Sums of squares	Degrees of freedom	Variance estimate
Between males	941	4	235.25
Within males	755	10	75.5
Total	1696	14	

Appendix T3.

Activity in the arena of outbred flies compared with that of the F1 progeny of a cross between two highly inbred lines.

Sample 1. Males.

Outbred flies		F1 progeny	
38	42	70	4
29	24	9	30
68	27	87	13
31	40	87	4
78	46	50	36
51	51	28	10
72	30	16	27
59	19	37	36
25	10	13	27
83	3	11	2
10	2	35	58
36	38	70	80
10	54	33	29
88	22	11	59
39	54	50	41

Sample 2. Females.

Outbred flies		F1 progeny	
20	55	15	64
39	45	33	38
19	10	4	41
10	13	24	50
25	24	27	50
27	37	18	26
11	33	25	59
37	17	20	40
20	13	14	7
14	33	27	76
34	13	29	23
42	25	35	5
22	43	17	54
25	42	11	31
17	23	33	10
20	27	28	48
4	24	16	39
17			

Appendix T5.

The percentage orientation exhibited by the F1 progeny of two crosses between inbred lines.

Cross 1	Cross 2
63	70
73	73
79	55
39	65
51	68
77	57
87	73
89	62
82	74
52	66
55	69
57	
60	
63	
49	

Appendix T7.

Activity in the arena under two different intensities of light.

Sample 1

	430 foot lamberts	4300 foot lamberts
	82	65
	93	88
	92	76
	87	80
	67	44
	80	55
	98	78
	70	72
	88	16
	63	97
Means	82.7	67.1

Item	Sum of squares	Degrees of freedom	Variance
Between samples	1217	1	1217
Within samples	6331	18	351.7

$F = 3.46 \qquad p = < 0.1$

Sample 2

430 foot lamberts	4300 foot lamberts
39	46
34	55
38	70
60	76
36	62
32	55
59	47
32	67
68	56
64	71
18	37
34	48
87	63
44	67
70	56
Means 47.7	58.4

Item	Sum of squares	Degrees of freedom	Variance
Between samples	864	1	864
Within samples	6739	28	240.7

$F = 3.59 \qquad p = < 0.1$

Sample 3

430 foot lamberts	4300 foot lamberts
41	55
63	72
52	69
70	47
45	87
28	76
39	51
17	61
1	47
46	82
35	73
41	85
80	101
42	69
65	52
Means 44.5	68.5

Item	Sum of squares	Degrees of freedom	Variance
Between samples	4368	1	4368
Within samples	9487	28	338.8

$F = 12.89 \qquad p = < 0.01$

Sample 4

430 foot lamberts	4300 foot lamberts
47	62
62	84
84	63
64	54
46	65
94	72
61	90
67	90
70	58
28	75
87	60
42	35
44	41
64	45
70	71
Means 64.3	62.0

Appendix T9.

Activity in the arena of flies reared in the cold compared with controls.

Sample 1

Control	reared at 18°C
111	87
87	72
94	58
53	90
60	90
85	70
60	1
73	99
93	79
93	50
112	76
98	27
94	87
73	72
92	86
Means 85.2	69.6

Item	Sum of squares	Degrees of freedom	Variance
Between samples	1825.2	1	1825.2
Within samples	14230.0	28	508.2

Sample 2

Control	reared at 18°C
85	95
78	101
85	73
95	29
67	84
58	71
53	58
86	79
82	102
65	70
80	76
55	85
87	80
77	39
90	98
76.2	76.0

Means

Appendix T10.

Courtship of flies reared at 18°C compared to flies reared at 26°C.

Control				'Cold reared' flies			
N*	% o.	% v.	% l.	N	% o.	% v.	% l.
45	57.8	28.9	13.3	114	43.0	38.0	19.0
25	44.0	36.0	20.0	37	51.4	35.1	13.5
94	74.5	20.2	5.3	88	76.1	13.6	10.3
83	60.2	30.1	9.7	27	50.8	25.9	23.3
36	58.3	27.8	13.9	58	75.9	17.2	6.9
20	55.0	30.0	15.0	59	50.8	33.9	15.3
101	53.0	34.0	13.0	48	39.6	45.8	14.6
47	68.1	27.7	4.2	66	77.3	22.7	0
98	40.0	47.0	13.0	83	59.0	32.5	8.5
47	62.8	31.9	5.3	29	62.1	20.7	17.2

*N - the number of units of courtship from which the percentages are calculated.

Appendix T11.

Activity, in the arena, of males at 16°C and at 26°C.

Sample 1				Sample 2			
16°C		26°C		16°C		26°C	
3	25	11	49	17	17	19	37
9	18	19	66	16	11	14	10
1	40	20	53	26	20	56	53
15	59	11	54	1	39	63	35
15	43	43	70	11	23	25	34
38	58	53	81	13	9	57	9
30	31	77	45	18	16	40	2
16	34	45	70	20	27	54	19
46	47	22	90	24	12	52	44
25	60	64	47	22	8	13	50
13	49	54	59	43	26	62	43
21	38	84	85	28	16	9	41
35		30		21		8	
M = 30.76		M = 52.08		M = 19.36		M = 33.96	

Q_{10} = 1.69 Q_{10} = 1.75

Appendix T12.

The courtship behaviour of 'environmentally small' males reared under crowded conditions compared with that of controls.

Environmentally small males

% orientation	% vibration	% licking
64	26	10
60	29	12
72	16	12
62	27	11
65	25	10
63	31	6
68	20	12
64	27	9
64	24	12
58	31	11
50	38	12
70	22	8
59	32	9
68	22	10
68	25	7
61	24	15
58	31	11
54	29	17
53	35	12
73	21	6

Appendix T14.

Single pair courtships of large and small selected line males with control females.

a. PAL males

No. of courtship movements	% orientation	% vibration	% licking
104	79	19	2
111	67	28	5
110	65	29	6
120	86	13	1
104	65	34	1
52	65	33	2
121	65	32	3
112	84	14	2
63	65	32	3
62	74	24	2
55	75	22	3
127	71	22	7
86	71	26	3
29	69	17	14
47	70	21	9
88	74	24	2
36	67	24	9
107	71	21	8
19	63	31	6
33	64	24	12

b. PBL males

No. of courtship movements	% orientation	% vibration	% licking
120	51	46	3
107	58	34	8
112	77	20	3
111	61	30	9
41	64	27	9
55	60	33	7
112	59	39	2
111	74	24	2
29	55	28	17
112	87	10	3
36	59	32	9
95	64	31	5
23	52	39	9
42	50	46	4
125	61	36	3
114	73	20	7
22	69	23	8
27	60	30	10
108	65	30	5
103	70	25	5

c. PAS males

No. of courtship movements	% orientation	% vibration	% licking
117	35	60	5
106	27	64	9
112	48	44	8
111	51	45	4
113	44	56	0
22	22	68	10
104	39	47	14
111	42	53	5
108	39	54	7
107	38	52	10
76	37	55	8
111	34	56	10
120	43	45	12
106	54	42	4
115	46	45	9
113	40	48	12
115	36	58	6
106	42	48	10
105	53	44	3
108	37	54	9

d. PBS males

No. of courtship movements	% orientation	% vibration	% licking
31	37	50	13
35	51	40	9
107	30	70	0
59	42	37	21
103	30	53	17
49	43	41	16
59	37	53	10
126	48	45	7
50	46	44	10
29	31	48	21
37	49	43	8
30	30	57	13
19	37	53	10
20	27	64	9
125	49	45	6
119	41	56	3
36	45	31	24
118	32	62	6
27	30	55	15
115	59	34	7

Control males

No. of courtship movements	% orientation	% vibration	% licking
116	60	28	12
54	41	49	10
103	44	47	9
75	47	43	10
79	86	10	4
95	57	24	9
74	43	47	10
47	47	38	15
48	58	37	5
23	74	13	13
21	52	43	5
65	78	17	5
22	27	55	18
30	33	56	11
115	45	51	4
25	44	52	4
45	74	17	19
35	40	49	11
37	49	38	13
108	70	26	4

Statistical comparison of the percentage vibration exhibited by the large and small selected males.

PAL and PAS

Source of variation	Sum of squares	Degrees of freedom	Variance estimate
Between samples	7507.6	1	7507.6
Within samples	1700.8	38	44.76

$F = 167.7$

$\therefore$ PAS males exhibit significantly more vibration than PAL males

$p < 0.1\%$

PAL and PBS

Source of variation	Sum of squares	Degrees of freedom	Variance estimate
Between samples	6117	1	6117
Within samples	2594	38	68.3

$F = 89.6$

$\therefore$ PBS males exhibit significantly more vibration than PAL males

$p < 0.1\%$

PBL and PBS

Source of variation	Sum of squares	Degrees of freedom	Variance estimate
Between samples	3572.1	1	3572.1
Within samples	3427.5	38	90.2

$F = 39.6$

$\therefore$ PBS males exhibit significantly more vibration than PBL males

$p < 0.1\%$

PBL and PAS

Source of variation	Sum of squares	Degrees of freedom	Variance estimate
Between samples	5629	1	5629
Within samples	1546	38	40.7

$F = 138.3$

$\therefore$ PAS males exhibit significantly more vibration than PBL males

$p < 0.1\%$

Appendix T15.

Single pair courtships of large and small selected line males courting their 'own' females.

No. of courtship movements	% orientation	% vibration	% licking
PAL			
121	64	25	11
93	77	19	4
142	68	24	8
100	66	31	3
38	63	34	3
PBL			
104	66	32	2
72	75	17	8
215	44	44	12
PAS			
177	22	72	6
131	36	54	10
180	23	67	10
152	16	70	14
25	40	52	8
150	29	58	13
117	41	46	13
82	33	59	8
47	38	45	17
PBS			
55	49	45	6
82	51	35	14
42	52	33	15
51	24	67	9

Appendix T16.

Correlation between thorax length and percentage orientation in the Pacific stock.

Sample 1		Sample 2	
Thorax length	% orientation	Thorax length	% orientation
55	50	60	57
58	72	61	69
53	60	58	41
61	67	57	60
57	76	57	62
60	49	55	60
60	49	56	51
56	60	50	50
53	25	58	58
		56	53
Means 57.0	56.4	56.8	56.1

Samples 1 and 2 combined. F test.

Item	Sum of Squares	Degrees of freedom	Variance
Total	1440	18	-
Regression	1.42	1	1.42
Remainder	1438.58	17	84.3

Remainder variance greater than regression variance; F value therefore not calculated. Regression not significant.

Sample 3

	Thorax length	% orientation
	50	79
	57	79
	52	55
	50	43
	55	55
	56	70
	53	38
	55	63
	52	54
	58	46
Means	53.8	58.2

F test.

Item	Sum of squares	Degrees of freedom	Variance
Total	1853.6	9	
Regression	0.718	1	0.718
Remainder	1852.882	8	231.6

Remainder variance greater than regression variance; F value therefore not calculated. Regression not significant.

Sample 4

Thorax length	% orientation
62	76
61	56
61	73
58	42
59	64
59	67
62	72
61	78
59	60
61	60
62	43
60	41
62	60
63	60
60	63
Means 60.7	61.0

F test.

Item	Sum of squares	Degrees of freedom	Variance
Total	1941.90	14	
Regression	2.18	1	2.18
Remainder	1939.72	13	149.21

Remainder variance greater than regression variance; F value therefore not calculated. Regression not significant.

Sample 5

Thorax length	% orientation
63	54
62	42
59	40
64	39
63	62
61	51
64	71
66	42
66	49
62	56
64	65
64	71
65	39
66	67
Means 63.5	53.4

F test.

Item	Sum of squares	Degrees of freedom	Variance
Total	1899.4	13	-
Regression	0.411	1	0.411
Remainder	1898.99	12	158.25

Remainder variance greater than regression variance; F value therefore not calculated. Regression not significant.

Appendix T17.

Activity, in the sector, of 100 flies (50 males and 50 females) from each line and control. The χ^2s were calculated by comparing the numbers of flies that had moved above and below the 4" radius.

Line	Distance covered			
	0	2"	4"	6"
Control	4	15	30	50
PAL	28	28	34	10
PBL	3	28	37	32
PAS	6	21	47	26
PBS	4	27	35	34

Appendix T19.

Activity, in the arena, of PVS, PVL and control males.

PVS		PVL		Control	
79	64	25	17	62	58
33	15	12	48	67	52
41	20	1	5	5	42
76	18	24	45	31	51
49	19	54	36	40	31
31	65	53	46	79	33
65	81	46	48	52	75
61	49	54	28	39	24
70	60	28	37	44	60
28	80	47	19	59	89
68	71	68	17	66	54
75	46	49	82	17	61
75	46	52	71	86	72
47	74	41	50	57	53
34	66	18	42	64	68
89	58	40	56	48	19
59	75	74	46	90	65
42	43	49	51	60	56
65	78	57	40	48	58
76	49	18	39	38	64

Item	Sum of squares	Degrees of freedom	Variance estimate
Between samples	3050.4	1	3050.4
Within samples	28174.6	78	361.2

$F = 8.4 \quad p < 0.01$

Appendix T20.

Single pair matings of HR, LR and control males with control females.

H.R.

% orientation	% vibration	% licking
57	32	11
55	31	14
51	36	13
59	26	15
57	31	12
72	21	7
65	27	8
57	32	11
58	27	15

L.R.

55	34	11
42	51	7
53	40	7
74	22	4
52	42	6
42	43	15
41	39	20
42	47	11
57	32	11

Control

63	30	7
60	31	9
72	23	5
72	20	8
72	22	6
68	27	5
58	27	15
63	29	8
69	22	9

F tests on % vibration

H.R. and Control

Item	Sum of squares	Degrees of freedom	Variance estimate
Between samples	56	1	56
Within samples	284	16	17.8

$F = 3.1$

L.R. and Control

Item	Sum of squares	Degrees of freedom	Variance estimate
Between samples	787	1	787
Within samples	725	17	45.3

$F = 17.4 \quad p < 0.001$

Appendix T23.

Activity, in the arena of A1, A2, I1, I2, and control flies.

	A1	A2	I1	I2	Control	A1	A2	I1	I2	Control
	116	114	13	93	97	70	84.8	71	55	59
	76	104	45	61	78	78	98	73	55	76
	53	61	101	90	62	35	28	106	47	54
	101	105	53	77	102	66	70	83	72	30
	101	72	48	72	97	77	64	60	74	24
	95	82	99	81	88	81	101	54	70	55
	75	57	58	75	101	75	92	87	75	64
	79	100	70	51	47	69	76	83	58	73
	44	103	90	89	83	81	98	94	54	62
	72	76	18	27	64	80	57	74	57	72
M=	81.2	87.4	59.5	71.6	81.9	71.2	76.8	79.1	61.7	56.9

Appendix T24.

Courtship behaviour of A2, I2 and control males.

I2 ♂♂ with I2 ♀♀

% orientation	% vibration	% licking	No. of breaks in courtship	Average length of breaks
29	50	21	2	10.5 secs.
53	33	14	0	0
57	35	8	1	21.0
44	46	10	0	0
46	42	12	0	0
69	21	10	0	0
53	34	13	0	0
44	43	13	0	0
70	19	11	0	0
50	37	13	0	0
45	42	13	1	4.5
40	44	16	0	0
29	58	13	0	0
41	39	20	0	0
43	41	16	0	0
33	55	12	0	0
42	51	7	3	10.5
46	38	16	0	0
46	44	10	1	4.5
52	34	14	1	4.5

A2 ♂♂ with A2 ♀♀

% orientation	% vibration	% licking	No. of breaks in courtship	Average length of breaks
76	18	6	0	0 secs.
76	22	2	2	9
54	42	4	2	7
78	18	4	2	17
58	30	12	1	9
69	26	5	1	10.5
74	17	9	3	14.5
61	30	9	7	8
75	22	3	1	4.5
71	26	3	1	4.5
64	28	8	0	0
54	30	16	1	10.5
64	27	9	1	6
63	25	12	2	4.5
80	14	6	2	6
69	23	8	4	7.5
61	31	8	0	0
80	16	4	0	0
67	30	3	0	0
79	15	6	2	4.5

Control ♂♂ with control ♀♀

% orientation	% vibration	% licking	No. of breaks in courtship	Average length of breaks
52	35	13	1	10.5 secs.
33	48	19	0	0
49	39	12	0	0
54	39	7	2	5.0
46	41	13	1	4.5
72	25	3	2	4.5
60	31	9	0	0
53	33	14	3	11.0
49	34	17	1	15.0
73	20	7	0	0

A2 ♂♂ with I2 ♀♀

% orientation	% vibration	% licking	No. of breaks in courtship	Average length of breaks
64	23	13	0	0 secs.
84	13	3	0	0
49	37	14	0	0
55	32	13	0	0
53	33	14	0	0
46	40	14	0	0
61	27	12	0	0
69	22	9	2	8.0
58	31	11	0	0
47	34	19	0	0
50	35	15	0	0
56	30	14	0	0
50	33	17	0	0
54	32	14	0	0
82	18	0	2	27.0

I2 ♂♂ with A2 ♀♀

% orientation	% vibration	% licking	No. of breaks in courtship	Average length of breaks
72	25	3	0	0 secs.
53	37	10	3	9
49	39	12	0	0
56	28	16	0	0
54	39	7	3	10.5
46	43	11	2	10.5
83	12	5	1	12.0
77	18	5	2	6.0
75	24	1	1	69.0
69	28	3	5	3.0
62	31	7	0	0
76	21	3	3	9.0
76	17	7	1	6.0
92	8	0	9	7.5
86	13	1	0	0

Appendix T26.

Activity, in the arena, of A3, A4, I3, I4 and control males.

A3	A4	I3	I4	Control
20	28	47	37	21
42	57	54	57	18
24	51	33	40	38
8	22	38	46	41
34	54	19	57	15
13	29	10	41	51
36	40	13	44	39
42	28	44	28	44
33	29	32	30	51
49	40	32	23	19
36	64	13	40	26
31	15	32	53	10
38	33	27	40	39
42	27	20	7	43
32	21	55	18	53
11	64	8	9	14
23	18	50	38	63
31	10	28	23	11
12	20	21	43	14
23	21	21	50	30
21	17	4	10	2
27	61	4	33	3
40	16	27	25	27
26	53	10	19	25
14	59	28	22	52
10	36	21	18	6
36	28	31	25	14
17	47	4	33	36
31	10	26	3	13
59	27	7	6	12

Appendix F7.

The results of mass mating experiments comparing males reared at 18°C and 26°C. All the females were reared at 26°C.

Time in minutes	Number of copulations			
	Sample 1		Sample 2	
	Cold reared flies	Controls	Cold reared flies	Controls
2	3	0	3	11
4	14	7	24	26
6	23	15	31	32
8	30	18	38	33
10	37	23	40	34
12	39	23	42	35
14	39	26	43	36
16	41	27	45	36
18	41	27	46	36

Appendix F12.

Correlation between thorax length and percentage orientation in the F2 progeny of crosses between the large (PAL and PBL) and small (PAS and PBS) selected lines.

% orientation	Thorax length	% orientation	Thorax length
51	65	63	70
53	65	67	70
54	66	68	70
25	67	42	71
51	67	53	71
67	67	71	71
69	67	76	71
84	67	81	71
53	68	48	72
54	68	54	72
63	68	63	72
73	68	79	72
43	69	59	73
49	69	60	73
70	69	61	73
77	69	65	73
32	70	69	75
50	70		

Item	Sum of squares	Degrees of freedom	Variance
Regression	6.63	1	6.63
Remainder	203.37	33	6.16
Total	210	34	-

F = 1.08

Appendix F13.

Percentage vibration in SRS and control lines.

SRS

F_1	F_3	F_5	F_7
34	47	41	45
38	49	50	59
50	43	53	45
27	33	53	44
34	37	55	47
32	57	47	51
40	44	50	37
	30	39	52
	38	53	62
	47	54	50

Control

41	22	29	44
31	32	35	43
32	31	30	28
40	26	26	37
27	27	39	41
44	31	30	19
	30	33	29
	40	26	35
		37	31
		38	34

Item	Sum of squares	Degrees of freedom	Variance estimate
Sum of regressions	13.4	2	
Combined regression	4.5	1	
Difference	8.9	1	8.9
Remainder	58.6	67	0.87

$F = 10.2 \qquad p < 0.01$

Appendix F13.

Percentage vibration in PVL and control lines during the 9 generations of selection.

PVL

F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
57	38	25	30	38	41	36	15
33	51	40	8	22	36	35	16
34	45	41	30	29	25	44	19
28	39	29	9	27	34	30	28
28	44	40	35	32	33	47	6
36	42	29	23	43	29	27	29
40	32	15	39	34	31	34	14
44	17	16	36	37	28	22	23
30	28	34	36	32	21	39	24
20	31	43	34	48	26	41	23
38	40	43	26	40	32	7	14
50	33	34	33	36	53	8	18
8	34	44	39	27	42	21	23
36	37	48	35	29	20	25	23
15	29	40	40	35	25	31	22

Control

F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
45	29	34	22	39	41	35	38
26	41	40	23	44	31	32	61
26	40	29	36	41	30	33	41
37	38	33	32	36	39	40	51
36	43	32	32	34	50	29	29
50	48	27	31	38	46	44	35
33	43	36	33	47	40	30	43
36	30	33	40	41	26	37	35
9	29	24	34	27	28	33	38
27	31	42	32	37	45	30	43
42	29	39	40	40	50	45	45
29	38	36	30	36	42	42	43
26	27	45	43	41	39	28	44
20	31	28	47	38	49	29	41
4	27	26	35	44	42	33	31

Item	Sum of squares	Degrees of freedom	Variance estimate
Sum of regressions	72.7	2	
Combined regression	25.6	1	
Difference	47.1	1	47.1
Remainder	1187.3	236	5.03

$F = 9.4 \qquad p < 0.01$

Appendix F14.

The results of mass mating experiments using HR, LR and Control males with control females.

Time in minutes	Sample 1			Sample 2			Sample 3		
	HR	LR	Control	HR	LR	Control	HR	LR	Control
2	2	2	4	7	1	5	1	3	1
4	19	18	15	27	14	25	21	12	11
6	33	29	28	41	22	39	35	18	24
8	37	33	33	44	25	42	42	20	29
10	41	35	37	48	27	46	45	23	33
12	42	37	38	49	28	47	47	25	35
14	44	39	38	49	29	47	48	27	39
16	45	39	38	49	30	48	48	28	41
18	45	40	38	49	31	48	48	29	42

Appendix F16.

Mass mating experiments done with flies with partially and almost completely amputated wings.

Time in minutes	Sample 1				Sample 2			
	Control	'B'	'C'	'A'	Control	'B'	'C'	'A'
2	0	1	0	0	1	0	1	0
4	8	5	4	1	3	2	1	0
6	17	13	7	3	5	7	3	0
8	22	18	10	5	8	7	4	1
10	28	23	13	8	8	8	4	1
12	30	25	17	9	10	9	5	2
14	33	27	18	10	11	9	6	2
16	35	30	18	11	11	9	6	2
18	35	30	19	11	12	10	6	2

ABSTRACT OF THESIS

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Degree Ph.D. *Date* 1st May, 1963.

Title of Thesis Some studies on genetical and environmental factors affecting the behaviour of Drosophila melanogaster.

Two aspects of the behaviour of Drosophila melanogaster were investigated, namely the courtship of the male and activity. The former is a useful pattern for genetical experiments as it is stereotyped, easily elicited and capable of being quantitatively analysed. The latter is a less precise measure but is important as its level will be affected by many factors, behavioural and otherwise.

It is known that both these behaviour patterns are subject to large environmentally induced fluctuations and I attempted to discover their cause. The results of these experiments were mainly negative and it was demonstrated for example that the fluctuations were not due to changes in temperature either during development of the flies or during testing or to changes in light intensity. A diurnal rhythm of mating speed was demonstrated which would increase within day variance. Howeve the fluctuations are mainly long term ones occurring between days and generations and measures of repeatability showed that over a period of a few hours the variance of activity and courtship measures was relativel small.

An attempt to separate the genotypic and environmental components of variance for activity and courtship was attempted but without success.

In order to examine the way in which the genotype controls behaviour two classes of experiments were carried out. In one, lines of flies which had already been selected for some morphological character were examined for changed behaviour patterns. In the other, I selected directly for changes in behaviour.

The courtship behaviour of lines of flies which had been selected for large and small body size was examined. Large selected males exhibited lower intensity courtship than those from the unselected

Use other side if necessary.

stock and the small selected line males, higher intensity courtship. It was experimentally demonstrated that the change in the courtship behaviour of the small selected males was due to a secondary selection pressure and that the increase in courtship intensity compensated for some deficiency in the small males' courtship which had resulted from the decrease in body size.

It was suggested that this courtship deficiency could, in part, be due to the fact that the smaller wings of these males was providing less sexual stimulation via vibration. A further series of experiments demonstrated that this was indeed possible as there is a positive correlation between wing area and sexual success.

Two attempts were made to change the levels of activity by selection. Neither experiment succeeded in this aim but nevertheless some interesting results were obtained. In the first selection experiment lines of flies were obtained which differed in their reactivity to one another. These changes were shown to have large effects on courtship behaviour. Flies from the highly reactive lines showed lowered courtship intensity and those from the unreactive lines, heightened courtship intensity.

The second selection experiments succeeded only in lowering 'activity' as it was measured in the selection apparatus. This selection response, however, was shown to be due not to a change in the level of activity but to the manner in which the flies responded to one aspect of the selection apparatus.

The relevance of these results on the evolution and organisation of behaviour patterns is duscussed.