EFFECTS OF ARTIFICIAL SELECTION

ON HABITAT PREFERENCE IN *DROSOPHILA*

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I hereby declare that this thesis has

been composed by myself, and that the

work it contains is my own.

Pierre Hutter

Abstract

This project concerns variation in preference for environmental variables in <u>Drosophila</u> and its relationship to habitat selection. Several lines of the two sibling species <u>Drosophila</u> <u>melanogaster</u> and <u>D</u>. <u>simulans</u> were therefore selected for both positive and negative preadult taxis in relation to three environmental variables supposedly relevant to <u>Drosophila</u> ecology. Pupation site preference for white light intensity as well as larval chemopreferences for ethanol and acetic acid were assessed in a gradient apparatus and the genetic architectures underlying these behaviours were investigated from the responses to artificial selection, reciprocal hybridizations between divergent lines and a chromosomal analysis. <u>D</u>. <u>simulans</u> responded only to selection for negative larval photopreference, while <u>D</u>. <u>melanogaster</u> responded strongly to selection for positive but weakly to selection for negative photopreferences. The results from ethanol preference experiments were not conclusive whereas those from acetic acid preference experiments suggested that in <u>D</u>. <u>melanogaster</u> natural selection might favour preference for low concentrations of this compound.

The possibility of environmental and/or genetic correlations between preadult and adult photopreferences

was examined in both species by recording the behaviours of gravid females of the selected lines at the time of oviposition. The results supported a genetic correlation between preadult and adult preferences, and the potential role of this correlation in enhancing habitat loyalty was considered.

The effect of the environmental conditions experienced by the flies immediately after their choice had been made was also examined. Selected lines of flies that experienced conditions grossly at variance with those they chose (traumatic lines) sometimes diverged in preference from those that experienced the conditions they had chosen (rewarded lines). The traumatic lines always diverged from the corresponding rewarded lines in the direction of preferences that were associated with high fitness, as revealed by low response to selection in the rewarded lines. Although the difference between traumatic and rewarded lines was stable under constant environmental conditions, the hereditary basis of the divergence seems to be complex and various possible interpretations of the results are discussed.

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Cette thèse est dédiée aux parents

d'Adam et Eve.

De mémoire de rose, on n'a jamais vu

mourir un jardinier.

(french saying)

x

CHAPTER 1 : GENERAL INTRODUCTION

1.1 - General background

The study of the Evolutionary Theory can legitimately be compared to the task of retracing back the course of a stream by collecting a few drops of its muddy water. Since evolutionary changes take place over relatively long periods of time, the average duration of research grants must be born in mind when considering the small amount of gold often found among the drops sampled.

In this "against-the-stream race" one of the firmest planks for research on the evolutionary mechanisms supposed to be at work in nature is to investigate how subpopulations stemming from the same base population may diverge in their genetic architecture when they are consistently exposed to different environmental conditions.

Natural environments vary in space and time, which can result in variable selective forces on the inhabiting populations. The main consequences of variable selection are then local adaptation (e.g. Clausen et al., 1940) and changes in gene frequency with time (e.g. Wright and Dobzhansky, 1946). In addition, both spatial and temporal variability of environment tend to allow the main-

tenance of genetic polymorphisms (Felsenstein, 1976 ;
Mackay, 1981), a preliminary condition for some mode of
population differentiation to occur. Therefore the ability
of animals to select a habitat appropriate for their own
genotype can be very important both in producing local
adaptation and in maintaining a high level of genetic
polymorphism.

1.2 - The importance of habitat selection in the main-
tenance of genetic variability

Waddington et al. (1954) already emphasized how
habitat preferences, if coupled with different fitness
coefficients in different environments could lead to the
appearance of a stable polymorphism in the population as a
whole. With the acquisition of mobility animals have become
more able to select places to explore, in which to settle,
and more importantly in which to leave their offspring.
Such a capacity is referred to as habitat selection which
can readily lead, as just mentioned, to substantial changes
in gene frequencies of subpopulations in different habitats
by exposing them to different selective regimes.

If animals integrate information about the pot-
ential suitability of alternative places in which to
settle, they would then be expected to select habitats in
which they have high fitness. Individuals indeed show re-
fined ability to assess their probable success, although

such apparent discrimination or active choice must be in-
terpreted very cautiously since several factors often
contribute simultaneously to explain the observed spatial
and temporal distribution of animals. Predators, parasit-
ism as well as inter- and intraspecific competition for
space or food are probably the best known such factors
involved in the control of the distribution of coexisting
populations in nature.

Nevertheless, it is the extent to which an active
choice of habitat can ultimately favour genetic polymorph-
ism which primarily interests us in the present context.
Several mathematical models have been proposed to account
for the maintenance of genetic polymorphism in heterogen-
eous environments, based on habitat selection linked to
fitness differences. For example Taylor (1976) argued that
whenever organisms practice habitat selection, genetic
polymorphism can be maintained under conditions much weak-
er than heterozygous advantage if the different genotypes
are able to select the ecological niches in which they are
most fit. Maynard Smith's (1966) model showed how dis-
ruptive selection operating on genotypes which control
host selection could be expected to ultimately lead to
sympatric speciation under favourable conditions.

The crucial question of whether a stable poly-
morphism can effectively lead to sympatric splitting of a
single population into two sexually isolated populations

has been a highly controversial subject for years. Yet the circumstances under which the preliminary situation favouring the establishment of a stable polymorphism on which disruptive selection can act is difficult to investigate in the field. It still remains very important to ask whether the presence of genes conferring a selective advantage in a particular "niche" often tends to be related to individual preferences for that "niche" since this would constitute a strong basis for an equilibrium state in which gene frequencies would remain constant but different in different habitats. Jones (1982) showed that in semi-natural conditions the land snail <u>Cepea</u> <u>nemoralis</u> indeed tends to display such association between genetic differences and behavioural differences. Different shell genotypes do not apparently have the same patterns of activity which might contribute to the maintenance of shell polymorphism by influencing individual fitness.

Several laboratory studies have shown that under relatively intense selection pressures subpopulations can be expected to diverge even when a maximum gene flow is allowed to occur (e. g. Pimentel <u>et</u> <u>al</u>., 1967). Nevertheless, the disruptive selection pattern evoked in such studies remains difficult to relate to field conditions and therefore the main debate still focuses on the nature of the barrier to gene flow between speciating populations. Mayr (1947, 1978) was one of the first to adopt the extreme view according to which there will be no possibil-

ity of speciation occuring as long as no assortative mating effectively prevents free hybridization between animals with different habitat preferences. Still Jones (1980) points out that habitat selection "might, nevertheless, lead to the genetic division of a population if the different genotypes mate within the micro-habitats which they have chosen", which explicitly suggests another way in which an assortative mating pattern can be guaranteed.

Although the proponents of sympatric speciation mostly presented theoretical models for sympatric speciation (e. g. Maynard Smith, 1966 ; Bush, 1975 ; White, 1978),there is now some good factual evidence to support it. Since the models were usually based on very few genetic changes underlying a speciation process their relevance seemed primarily restricted to host-specific, monophagous or parasitic animals. Nevertheless,habitat choice linked to genetic differences has indeed been described for a variety of genetic polymorphisms (Jones, 1980). Tauber and Tauber (1977) give some evidence that sympatric speciation can be at work in an interbreeding natural population of Chrysopa, conformably to the predictions of Maynard Smith's (1966) model, through selection on genes controlling diapause.

However the prevalence of such a mode of speciation relative to allopatric modes is in insects by no

means substantiated by empirical evidence (see Futuyma and Mayer, 1980, for a discussion). These authors stressed that the occurrence of oviposition preference (even among phytophagous insects) determined by experience as larva does not appear to be a widespread phenomenon at all in this group. As we shall see later my work initially tended to focus on further investigation of this possibility of conditioning effect or genetic correlation between preadult and adult preferences since I felt less sceptical than the above authors about the recent evidence from studies on stenophagous insects.

1.3 - Phytophagous insects and sympatric speciation

When considering the potential opportunities that food substrates offer to animals for self selection of a feeding site it appears that phytophagous insects occupy a privileged situation. They are therefore excellent candidates to exploit a wide range of spatially and temporarily variable surrounding conditions which in some cases can be expected to limit substantially the gene flow between incipient isolated subpopulations. Dethier (1954) noticed that diet specializations have reached their highest development among parasitic forms and the insects which feed upon plants, that is to say about half of the living insect species. Although he clearly asserts that in "no other groups of animals are feeding preferences so sharply delineated", the author points out that

despite the apparent specialization the observed preferential feeding is not primarily directed by nutritional requirements. Several behavioural factors as well as toxic principles present in non-chosen substrates largely contribute to influence such preferences. This indicates then that the way in which an apparently selected feeding site can guarantee high fitness is often delicate to establish.

Successful colonization of a new plant requires both behavioural adaptation such as oviposition site preference and physiological adaptation such as assimilation efficiency or the capacity to overcome toxic compounds. One can thus legitimately ask whether these traits tend to evolve to a coadapted state among phytophagous insects (Wasserman and Futuyma, 1981). These authors studied such a possibility in the beetle Callosobruchus maculatus but their results did not support such a coadaptation pattern. Results of selection and choice experiments indicated that the pigeon pea was a better host than the azuki bean while females preferred to oviposit on azuki bean. Increased capacity of the larvae to develop, survive and produce fertile offspring in one type of bean thus did not correlate with the tendency for females to oviposit on the same type of bean. The authors conclude that their study suggests "that the diets of phytophagous insects could be evolutionarily more labile at the behavioural level than at the physiological level".

Wood (1980) and Guttman et al. (1981) among
others documented that several biological differences exist
among conspecific insects native to different host plant
species. Guttman et al. (1981) collected nymphs of
Enchenopa binotata in a small area where individuals were
removed from seven host plant species to be analysed
electrophoretically. The results clearly demonstrated a
higher amount of genetic differentiation among host races
than within host races. Various ecological and ethological
differences were parallely observed between animals from
different host races and showed that gene flow between
host races was severely restricted despite insects in-
habiting adjacent tree species, even with inter-meshed
branches. As suggested by the authors these results sup-
port the idea that differential selection regimes alone
could not account for the apparition of separate gene
pools. Besides, limited gene flow among insects native to
different host species led to the differentiation of re-
productively isolated species along host plant lines.

Holometabolous insects are of particular in-
terest among phytophagous insects since the choice of a
pupation site where metamorphosis takes place irreversibly
determines the environmental conditions to which the de-
veloping imagos are exposed. Whatever factor primarily
underlies a given preference per se, the choice of a
typical pupation site by a larva (as well as a resting or
oviposition site by an adult) always implies that a pre-

cise microclimate or a set of biotic factors have to be accepted together. This logically brings us to the delicate question of what factors determine preferences, which can only be examined very superficially. Accurate preference patterns in insects are obviously of crucial importance to insure their close adaptation to local conditions which are usually highly changeable through seasonal cycles. Before it even identifies at close range a particular vegetable by its odor, taste, toxins or nutrient an insect can presumably respond to environmental variables such as temperature, humidity or light intensity to increase its chances of locating a suitable site. In other words any refinements which improve vision, phototaxis, geotaxis and chemotaxis may well contribute to guide an insect more effectively to places where its fitness can be maximised.

1.4 - The concept of preference

It is worth realizing first how huge an abstraction the concept of preference clearly is, used in this context only as a semantic device necessary to describe the observations made of a correlation between some activity of an organism and a component of the external world. Moreover the investigation of the physical basis accounting for a preference and the physiological mechanisms involved in its expression is often made difficult by the possible implication of the central nervous system

in mediating the behaviour.

Many of the behavioural traits which mediate a preference can quite simply be accounted for by straightforward causes such as the presence or absence of some peripheral sensory structure (such as a modification of the antennae) or even of a single muscle allowing a particular physical performance. For instance Thomas and Wyman (1982) showed that in Drosophila melanogaster the absence of the tergo-trochanter muscle (due to a single X-linked mutation) prevents the expression of the escape-jump response, which presumably also restricts the access to certain sites otherwise more sought for.

Nevertheless, in many cases the contribution of the central nervous system to both the quality and/or the quantity of the expression of a so called preference cannot be denied. Just as a spider monkey spends most of its time in the trees for reason undoubtedly due to something in the neural and psychic activity of its brain (Williams, 1974) it can be argued that habitat preferences in invertebrates can be influenced by some brain activity. Among the infinite range of stimuli which surround an organism, only part can have a significant impact on its sense organs, out of which only a filtered proportion is processed by the brain to produce an extraordinarily simplified representation or "map" (made up by the overall result of the integration of the external impact) of the external

world. Each species then obviously uses its own sensory equipment and its specific "map" which enable it to perceive at least the environmental clues most useful to its survival. The complicated process separating the gathering of environmental information through sense organs from the phenotypic expression of a behavioural response of a preference certainly makes the task of visualizing the nature of the correspondance stimulus-behaviour highly speculative.

Moreover the "choices" we commonly observe among animals, to which we are inclined to assign a unique preference value are not simple permanent or fixed behaviours. Gould and Gould (1983) insist that when honey bees, show spontaneous preferences for certain colours and shapes of flowers such display of preference is not absolute, but probabilistic. Given a choice between two alternatives we know from discrimination tests it can distinguish reliably an animal chooses its "favourite" most of the time but not all the time.

1.5 - Habitat preference in Drosophila

It is no surprise that within the extensively studied insect genus Drosophila there have been a number of field investigations aimed at a better understanding of various aspects of the mechanism of habitat selection. Taylor and Powel (1978) were able to show by mark-release

recapture experiments that <u>Drosophila</u> <u>pseudoobscura</u> and <u>D</u>. <u>persimilis</u> tend to return to their area of original capture or an area ecologically similar to it, although a genetic basis for this variation was not proven. Jungen and Wunderlich (1972) observed that in <u>D</u>. <u>subobscura</u> gene arrangement frequencies differ among flies caught early in the evening and those caught close to dark, which suggests that flies carrying various gene arrangements differ in their use of different microhabitats. In his review of habitat selection in <u>Drosophila</u>, Parsons (1978) generalizes that "adults are able to distribute themselves into microhabitats suitable to their ecological requirements, the main controlling factors being wind intensity, humidity, temperature, light intensity, food sources, and acceptable courting and oviposition sites". The study by Atkinson and Shorrocks (1977) clearly shows how specific are the breeding sites chosen by the domestic species of <u>Drosophila</u>.

1.6 - <u>Environmental components and organisms used for the</u> <u>artificial selection for preferences</u>

As we have seen, the precise way in which habitat choice operates in the wild is difficult to elucidate since numerous environmental variables with which animals continuously interact cannot be studied individually. Even when parallel laboratory studies are conducted in order to investigate preferences along a gradient of a single variable controlled experimentally, the interpretation of such

observations remains difficult. It is for instance often arguable that an organism basically goes indiscriminately anywhere until some deficiency in some stimulus causes a slowing down of an exploratory behaviour or more intense activity at such limit point. The simplest kind of orientation behaviour, the <u>kinesis</u> occurs whenever an animal is subjected to an unpleasant stimulus which is non-directional (Roberts, 1971). Fraenkel and Gunn (1940) argued that a variation in forward movement (what they called ortho-kinesis) depending on the intensity of stimulation by an environmental variable often explains the tendency that animals may show to aggregate in particular places. However the orientation preference behaviours I shall be dealing with in this study correspond more closely to the concept of a <u>taxis</u>, which is one type of self orientation with respect to a directional stimulus (Roberts, 1971). Additionally, it must be born in mind that whenever a mean preference is observed experimentally for a group of organisms presented with a gradient of a particular variable, this tells us, as a Partridge (1978) stressed that such a variable can be important in nature but it does not tell which cues the animals actually use to distribute themselves. More generally one has to reckon that the relation between the proximate and ultimate properties of any environmental element sought for or selected remains in most instances an unsolved problem.

Since one of the main purpose of my study was aimed at investigating how preferences displayed early in development are genetically controlled and environmentally influenced during development I firstly needed some standardized experimental design which could reliably provide an estimate of the additive genetic variation that natural populations harbour for the traits investigated.

Artificial selection experiments are often very informative about the genetic architecture of a behavioural trait as long as the trait selected can be rigorously controlled. For this purpose preferences for white light intensity seemed to be an appropriate environmental variable to start with as Waddington et al. (1954) for instance reported that various wild type and mutant stocks of Drosophila showed more variation in luminosity preferences than with other environmental variables, although these were adult preferences. Furthermore light has been widely used over the last two decades as repelling-attracting cue to compare adult photopreferences between Drosophila species through directional artificial selection so that the subject is well enough documented. Besides, light intensity preference of late larvae of Drosophila are easier to control experimentally than preferences for the two variables closely related to light in the field, namely temperature and humidity, or for any chemical occurring in the culture medium. This is because during selection for pupation site preferences in a stepwise light gradient

it is not necessary to force the larvae to pupate within the medium or on its surface. When the variable used is part of the medium itself, there is no means by which the pupation site preferences of all larvae present can be assessed as accurately as with incident light, since a proportion of the larvae always pupate outside the medium. Although artificial selection was always carried out for larval photopreferences, several behavioural observations were often made in parallel on adults in order to investigate the possibility of a correlation between preadult and adult phototaxis.

Experiments similar to those on light preferences were later carried out on earlier larval preferences for chemical compounds common in places where Drosophila are found, namely ethanol and acetic acid.

The use of Drosophila throughout the experiments was motivated by three main advantages these animals present with respect to my specific interest :

a) The presence of a well developed nervous system.

b) The experimental advantage of a short generation time.

c) The availability of balancer stocks carrying marker genes which allows detailed genetic analyses.

Apart from the genetic information <u>sensu stricto</u> the tremendous wealth of literature now available on almost every aspect of the biology of <u>Drosophila</u> gives this genus a privileged position among the other favourite organisms commonly used in genetics (microorganisms, nematodes or mice). In this study the two cosmopolitan sibling species <u>Drosophila melanogaster</u> and <u>Drosophila simulans</u> have been used for reasons which will become clearer through my literature review on phototaxis in this genus.

1.7 - Preadult and adult preference in insects

1.7.1 - Correlation between preadult and adult preferences

I have already referred to the idea of habitat loyalty whereby parallel trends in preferences, both in direction and/or in strength can be observed between preadult and adult insects. Two possible causes of this need to be distinguished, one environmental through direct conditioning and one through genetic correlation. The latter is chiefly due to pleiotropy (when a gene has the property to affect two or more characters) although it can occasionally result from genetic linkage.

For the investigation of such characteristics holometabolous insects with indirect development (showing complete metamorphosis), such as <u>Drosophila</u>, are of particular interest since the oviposition site preferences of the adults determine quite strongly the sort of micro-

environment to which all preimaginal stages of their off-spring will be exposed. Both larvae and pupae are indeed considerably restricted by their limited mobility to the conditions imposed by the substrate selected by their mothers at the time of oviposition. Carson (1971) in his review on the separation between feeding and breeding sites in Drosophila, points out how oviposition is for most species a very specialized and delicately adapted performance. In an experiment choice situation Bos and Boerema (1980) showed that when five different media were presented to the six D. melanogaster subgroup species so far described these proved to have highly specific pre-ferences for oviposition.

Mechanisms which would link adult behaviour to some early experience as preadult are probably more diff-icult to understand in organisms with more direct develop-ment, experiencing more diverse environmental conditions, even though this phenomenon has been better investigated in higher vertebrates than in insects. Partridge (1978, and references therein) reports positive effects of early experience of a habitat on later preference for that habitat in some insects, fish, amphibians, birds and mammals. Although amphibians have indirect development too, this represents a less drastic metamorphosis than in holometabolous insects.

Numerous attempts have been aimed at testing in polyphagous insects what Hopkins (1917) enunciated as the "Hopkins Host Selection Principle" which focussed on the possibility that some sort of memory of larval feeding habits could predispose them to oviposit as adults on the same species of plant as that upon which they had fed. From a theoretical point of view preimaginal conditioning could be expected to have long lasting effects either through habituation or associative learning on a trial and error principle. A form of olfactory preimaginal habituation which persists up to the adult stage has been reported in Drosophila melanogaster by Thorpe (1938), Manning (1967) and others. Flies are normally aversive to the smell of peppermint oil but when they are reared as larvae in a medium containing 0.5 percent peppermint oil they show greatly reduced aversion to it. Attempts to demonstrate associative learning in adult Drosophila and other Diptera have met with some success but in larvae Aceves-Piña and Quinn (1979) showed that a relatively rapid memory decay (less than 30 minutes) makes it very unlikely that conditioning could affect adult behaviour days later through memory.

1.7.2 - Possible correlation between preadult and adult photopreferences in Drosophila

It was tempting from my own study on early preferences for light intensity to look at whether the same

genes affecting larval light preferences could be responsible for the variation in adult preferences for light conditions at the time of ovipositing. This was investigated both in <u>D</u>. <u>simulans</u> and <u>D</u>. <u>melanogaster</u>,although a precise partitioning of the measured variance into environmental and genetic components was made only for the latter.

If any commonalties existed between components of the larval and adult visually based responses it is fundamental to work out first if they explain some possible constancy recorded in phototactic preference at the different stages. There is no <u>a</u> <u>priori</u> reason to believe that this can be the case in <u>Drosophila</u> since larvae of insects with complete metamorphosis lack both compound eyes and ocelli but may have laterally positioned photoreceptors called stemmata. Besides, Truman (1976) emphasized how larval extraretinal photoreception even plays the major role in the coordination of physiological and behavioural processes with daily and seasonal photoperiodic cycles. The cerebral lobe area of the brain is very likely to be the site of photoreception which mediates these rythms through neuroendocrine activity.

In <u>Drosophila</u> Demerec (1950) pointed out that if larvae lack compound eyes they still possess non-image forming photoreceptors at their anterior ends. However late third instar larvae already possess a partially developed compound eye and this seemed of some interest

since I precisely intended to record light preference at
this developmental stage. Waddington and Perry (1960)
observed that in D. melanogaster "the developing eye, un-
like certain other organs such as legs and wings, conti-
nues rather steadily and without interruption throughout
the prepupal instar and the first stages of the true pu-
pal period". They pointed out that twenty-four hours
after pupariation the various elements in the eye can be
easily recognized. More recent work is summarized by
Kankel et al. (1980) who report that during the middle of
the third instar larva the morphogenesis of the optic
lobes of D. melanogaster proceeds in conjunction with the
morphogenesis of the compound eye and "by the end of the
larval period the rudiments of the majority of the com-
ponents of the adult central nervous system are recogniz-
able". By the late third instar larva the eye imaginal
discs "occupy positions anterior and lateral to the brain
and are connected to the latter via the optic stalk".
Judging from Hanson's work (in Kankel et al., 1980) the
arrival of the first photoreceptor axon bundles to the
developing lamina takes place about twenty-four hours be-
fore pupariation.

In spite of the above considerations Markow
(1981) considers that genes controlling the structure of
the compound eye should not be expected to be functioning
in the larval photoreceptor system. She found nonetheless
that severe mutations at the norp A locus appear to leave

larvae blind as well as adults. This led her to suggest that at least part of the phototransduction process might be the same in both larval and adult photoreceptors. Although there are some discrepancies between descriptions of the formation centers of the optic lobes (see for instance White and Kankel, 1978 ; Hanson, 1978), this subject is currently under intense investigation and should soon be clarified by further study.

Before attempting to justify the experimental design I devised, it is necessary to report some of the laboratory work done to date since the study of phototaxis has been the concern of scientists working in quite different fields since it was in its infancy at the beginning of the century.

1.8 - Phototaxis in Drosophila

1.8.1 - Previous studies on Drosophila phototaxis

Most of the pioneer work on phototaxis in adult Drosophila was concerned with the study of the effects of white eye and bar mutant genes on photactic response (Brown and Hall, 1936 ; Scott, 1943). The results indicated that these genes determined altered phototaxis by their transformation of the reception area of the compound eye and therefore the neural input to the central nervous system was directly affected.

This line of investigation has been substantially developed by the use of gynandromorphs (sex mosaics) leading to a genetic dissection of phototactic behaviour (Benzer, 1967, 1973 ; Hotta and Benzer, 1970, 1972). Mosaic flies were produced by crossing males from a stock carrying on their X- chromosome a recessive marker gene (linked to the mutant gene X_M under study) to females from a stock having one of their X- chromosome ring shaped, X_R. This X_R is so unstable that it may get lost during the first division of zygotic females carrying it thus giving rise to flies with different genotypes in various parts of their body. The mutant was then only expressed phenotypically in male parts X_M and not in female parts $X_R X_M$.

This elegant technique enabled the authors to pinpoint the primary site of action of some radiation- and chemically induced mutations that they were studying. Results from such studies confirmed that conditions intrinsic to the eye are at least responsible for the presence or absence of phototactic behaviour. By tagging the genotypes of the eyes by colour genes, Hotta and Benzer (1972) studied a series of mutants having defective vision and abnormalities in the electrical response of the eye (ERG). While genetically normal eyes functioned normally the authors found that every mutant eye produced a mutant ERG.

More investigation on the variability of photo-
taxis and its genetic basis was later carried out using
selection experiments. This has been done mainly by using
Hirsch-Hadler multiple unit classification photomaze
(Hadler, 1964a) where flies entering the maze have to make
a series of fifteen successive light/dark choices, emerg-
ing into collecting tubes which are ranked in order of the
proportion of + and - choices made in the maze. Negative-
ly and positively phototactic strains have been success-
fully selected this way in D. melanogaster (Hadler, 1964a,
b, ; Walton, 1970 ; Markow, 1975 a, b), in D. simulans
(Markow, 1977), in D. ananassae (Markow and Smith, 1979),
in D. persimilis (Polivanov, 1975), in D. pseudoobscura
(Dobzhansky and Spassky, 1967, 1969 ; Woolf, 1972) and in
D. subobscura (Kekić and Marinković, 1974). Values for the
realized heritability of phototaxis found in these exper-
iments vary between less than 0.1 in D. pseudoobscura to
more than 0.5 in D. melanogaster.

1.8.2 - Importance of the experimental design

Rockwell and Seiger (1973) discuss three differ-
ent types of phototactic measurements which have been used
in laboratory studies. They point out that these three
designs do not necessarily measure the same type of
response to light :

a) Designs in which the measurement of photo-
taxis is a function of movement towards a directed

light source (light gradient parallel to the plane of movement of the organisms).

b) Designs in which the measurement of photo-taxis is done by the distribution of the organisms in a light field (light perpendicular to the plane of movement of the organisms).

c) Designs in which the measurement is done after the animals have repeatedly selected one of two alternatives at choice points, as in the phototactic maze described above.

The investigation of larval phototaxis was done with two designs which both fall into the second class, whereas that used to investigate adult phototaxis falls into the third class. Designs of the second class were thought to better simulate the natural situation in which larvae may often be exposed to a wide range of light intensities at the surface of their feeding substrates (such as a rotten fruit or vegetable). In a third instar larva ready to pupate, it can be assumed that such substrates expose one side of the larva to incident light, whereas its other side may be quite shaded.

Rockwell and Seiger (1973) point out that the sign and intensity of the phototactic response may be influenced by numerous factors such as temperature, age,

the effect of diurnal rythm, water balance or nutritional level. The first aim of my apparatus for measuring larval phototaxis was to avoid as much as possible the inter-ference between each trial of such factors in order to make sure that the recorded behaviour best reflected move-ment in relation to the gradient of light. This is indeed in accord with the definition of phototaxis in its broad sense even though several researchers have proposed more restrictive definitions. Regrettably many of these defin-itions are not very satisfactory when one considers the difficulty of using direct comparisons between different test procedures. Some of such discrepancies might reflect no more than various interests of separate researchers working in their specific contexts.

1.8.3 - Phototaxis in Drosophila in nature

Attempts to relate laboratory observations to field conditions must always take into account how a phototactic response is temperature and/or humidity de-pendent. In nature areas with high light intensities are usually associated with more dry microclimatic conditions. Médioni (1962) found that northern populations of $\underline{D}$. $\underline{me}$-$\underline{lanogaster}$ have a more positive phototaxis than southern ones. This seems in accordance with the expectation that under conditions of high temperature and dessication flies tend to select more damp habitats which are presumably more shaded. Kekić et al. (1980) report that progeny of

D. subobscura captured in neighbouring areas characteriz-
ed by contrasted light intensity had significantly differ-
ent scores when run through a photomaze (the flies from
the light areas being more photopositive).

The difference in photopreference between D. me-
lanogaster and D. simulans as measured in laboratory con-
ditions (see later) is worth to be compared with some
ecological differences that determine the divergence in
ecological niches between the two sibling species. Basden
(1954) noticed that D. simulans disappears completely in
Scotland at the end of November one month before D. mela-
nogaster does so. Kawanishi and Watanabe (1978) point out
that the absence of D. simulans inside houses has been re-
ported by Okada (1971) and Watanabe and Kawanishi (1976),
whereas D. melanogaster was found both inside and outside
houses. These authors suppose that the difference in
photopreference plays a role in the ecological different-
iation of the two species.

Interestingly enough many authors have found
that D. melanogaster was generally competitively superior
to D. simulans in the laboratory and was therefore expect-
ed to have a larger "potential niche breath". Still
Hoenigsberg (1968), Tantawy et al. (1970), Watanabe and
Kawanishi (1976) and Sokolowsky and Hansell (1983) report
striking observations which apparently support the view
that over the last two decades D. melanogaster tends to

loose ground in California, Columbia, Egypt and Japan in
competition with D. simulans.

1.9 - Chemotaxis in Drosophila

Mostly for the practical reasons alluded to
earlier on much less importance was given in this study
to the other environmental variables used for the invest-
igation of early preferences, ethanol and acetic acid.
Both these compounds are resources highly available where
D. melanogaster and D. simulans are found and the amount
of research carried out on enzyme activity at the alcohol
dehydrogenase (ADH) locus testifies the importance of this
product for the metabolism of the flies. Parsons and
Spence (1981) demonstrated that acetic acid acts as an
attractant to larvae of D. melanogaster at concentrations
down to 1/1000 of concentrations at which ethanol is
attractive. Thus if ethanol can be considered to be pri-
marily a food resource compound, acetic acid can be
thought to act as a recognition compound as well as a food
resource. The authors suggest that acetic acid acts as an
attractant similary for larvae and for adults of D. mela-
nogaster since their results on preadults paralleled very
closely the results of Fuyama (1976) on adults.

As can be predicted by numerous studies on
adult preference, larvae of D. melanogaster show strong
preference for alcohol containing media while larvae of

<u>D</u>. <u>simulans</u> do not (Parsons, 1977). Larval preferences for alcohol were studied in five <u>Drosophila</u> species by Gelfand and McDonald (1983) who found important inter-specific variation. Furthermore McKenzie and Parsons (1972) found some evidence that oviposition preference with respect to alcohol tends to parallel larval preference.

Additionally,it is worth considering to what extent pupae and adults can be sensitive to the vapours of acetic acid and alcohol. Van Herrewege and David (1978) gave strong evidence that a significant part of adult <u>D</u>. <u>melanogaster</u> nutrition can occur through their inhaling alcohol vapours. Similar results have been obtained in various species of <u>Drosophila</u> by Starmer <u>et</u> <u>al</u>. (1977) and Parsons <u>et</u> <u>al</u>. (1979). The authors believe that the vapours entering via trachae dissolve directly in haemolymph without having to cross the intestinal barrier.

1.10 - <u>Possibility that preference behaviour might be affected by environmentally induced cytoplasmic effect similar to dauermodifications</u>

This introduction focussed so far on the potential role of variability of individual preferences for particular environmental components acting as a trigger for or reinforcer of population differentiation. The ultimate genetic modifications that such phenomenon can bring

about in the incipient divergence of subpopulations or
demes is then entirely concordant with the orthodox neo-
darwinian approach in which genome-environment interactions
can be completely described in terms of differential
selective regimes.

At this point I nevertheless encounter some
difficulty in presenting much relevant background material
to justify some of the experiments described later since
one basis of my work was a hypothesis with almost no
empirical evidence to support it. In short I wanted to
ask whether harsh environmental stress could in extreme
cases alter some extranuclear hereditary factors maybe
involved in the control of early preference behaviours.
The theoretical possibility of such effects could presum-
ably be somehow related to those experiments reported on
the role of environmentally induced persistent changes
known as "Dauermodifikationen" (Jollos, 1935), whose liter-
al translation is "lasting changes". Grun (1976) reports
that "a number of experiments carried out using
plants, animals, and protozoa have shown that sometimes a
change can be induced, apparently by an environmental
factor, that is inherited through the maternal parent"...
and "persists, sometimes, through five or more gener-
ations."

Jollos (1935) who first discovered these so
called dauermodifications observed that by exposing D.

melanogaster late larvae to a twenty-two hour heat treat-
ment of 36°C he could obtain dwarf progeny of both sexes.
When these were repeatedly crossed together at 21°C the
proportion of dwarf individuals slowly decreased but the
author still found abnormally small flies up to the fifth
generation. Interestingly only progeny from the crosses
dwarf females x normal males produced some dwarf offspring
which led to the conclusion that cytoplasmic inheritance
was probably involved in the phenomenon. The author also
observed that late larvae exposed to a twenty-four hour
heat treatment of 36°C equally produced flies with ab-
normal abdomen or aeroplanoid wings showing a similar in-
heritance pattern. Flies with abnormal abdomen were even
found up to the sixth generation of untreated progeny.

All dauermodifications described in the liter-
ature deal with morphological characteristics so that
there is an important difference between these results
and my own concern as far as this deals with behavioural
characteristics. The only possible empirical evidence to
support the idea that the above phenomenon could also
apply to behaviour was found in a reinterpretation of
Sokal's (1966) data relative to central and peripheral
pupation site preferences of D. melanogaster larvae rear-
ed in shell vials. The author carried out various cross-
ing experiments between divergent lines selected for
each pupation site preference, and obtained results often
difficult to interpret, some of them even suggesting an

apparent "paternal" influence. Taking into account the
genetic architecture of the traits investigated it can be
argued that pupae exposed to the "peripheral situation"
were repeatedly experiencing more adverse environmental
conditions (as will be extensively discussed in chapter
5) than those pupae exposed to the "central situation".
The idea behind this was that an environmentally induced
cytoplasmic change (similar to the dauermodification phe-
nomenon) could thus be postulated to occur under somehow
thwarting circumstances, which seemed to provide an in-
teresting explanation in this particular example.

The reason why I initially felt that early pre-
ference behaviour could be a favourable place to further
investigate such phenomenon could be resumed as follows,
even though it may seem a little obscure at this stage.
If a preference behaviour pattern could be in some way
mediated by a cytoplasmic factor, such factor might be-
come of selective advantage so long as the exposure to
the environmental variable considered could simultaneous-
ly affect the action of the factor itself. This would be
so because a cytoplasmic susceptibility to interfere with
the environment at a particular developmental stage could
ultimately allow a greater genetic variance of the be-
haviour by differently mediating the expression of the
preference in different individuals. As a corollary,
circumstances under which such cytoplasmic factor could
show a capacity of self replication are expected to make

such an environmentally induced preference polymorphism
more likely to be promoted.

1.11 - Statement of aims of the present thesis

The composite nature of the present introduction
partly reflects the scope of the subject dealt with in
this thesis. Some points only briefly mentioned so far
will need to be more extensively discussed after the de-
scription of the experiments and their results. The basic
scheme of the experiments can then be summarized in the
following way.

1) An investigation of the genetic basis of
larval preferences in D. melanogaster and D. simulans,
firstly for light intensity and secondly for ethanol and
acetic acid. This was performed through artificial selec-
tion, reciprocal hybridizations of divergent strains as
well as a straightforward chromosome analysis in D. me-
lanogaster.

2) An investigation of adult photopreferences
in the same species aimed at examining the possibility of
environmental and/or genetic correlation(s) between pre-
adult and adult behaviours.

3) An investigation of the possibility that
some lingering alteration of the above preferences can be

induced by varying the levels of light and chemicals in a way related to what is suggested from observations on dauermodifications.

This last part of the work became an increasing preoccupation as the experiments progressed and therefore the concluding general discussion will pay particular attention to it.

CHAPTER 2 : PHOTOPREFERENCES IN D. SIMULANS

2.1 - Introduction

2.1.1 - Peculiarity of D. simulans phototaxis

The reason why D. simulans was used for the first set of experiments on phototaxis primarily stemmed from previous results relative to the influence of light on mating success. By looking at adult behaviour, Wallace and Dobzhansky (1946), Spieth and Hsu (1950), and Grossfield (1966, 1968, 1970) showed that light has an important influence on mating ability in Drosophila. Grossfield (1970, 1971) considers that Drosophila species fall into three main classes with respect to their dependence on light for mating. D. simulans belongs to the second or intermediate class, showing a significant inhibition of its mating ability in darkness. The genetic architecture underlying this ability can therefore be expected to be more flexible than that of members of the two extreme classes which are completely light dependent or light independent. Still the extent to which light directly affects behavioural traits involved in the structure of

courtship remains unclear. Grossfield (1971) pointed out the salient and rather unique situation of D. simulans. Most often cosmopolitan species are light independent for their mating ability like D. melanogaster and this difference initially seemed to me of some interest. McDonald and Parsons (1973) showed that the dispersal activity of D. simulans adults is also more dependent on the presence of light than in D. melanogaster.

2.1.2 - Previous results on phototaxis in D. simulans

D. simulans was useful material also because its phototactic behaviour is well documented both as larva and adult. Photomaze results support a polygenic, additive mode of inheritance for its level of photopreference as in all species examined so far (see Markow and Smith, 1979). The emerging picture for D. simulans adults is that of variance in phototaxis being controlled mainly by autosomal genes, while strongly sex-linked in D. melanogaster (Hadler, 1964b; Walton, 1970 ; Markow, 1975b, and Markow and Smith, 1977). Interestingly, more photopositive adult preference has been repeatedly observed in D. simulans (when compared with D. melanogaster) even with the use of quite different experimental designs (see also McDonald and Parsons, 1973, and Parsons, 1975). Kawanishi and Watanabe (1978) measured adult photopreferences of the two species in an apparatus presenting a gradient of light intensities. Although an "aggregation effect" of

the flies run simultaneously was not eliminated,the authors could confirm the above difference and their results support the common view that in experimental conditions D. simulans invariably prefers to stay and lay eggs in light places whereas D. melanogaster does not show such strong preference. Vaysse and Médioni (1982) report that the latter species shows two activity peaks (one at the beginning and the other at the end of the light period), while D. simulans has a single activity peak at about the middle of the light period, which seems in agreement with the above observations.

Remarkably the same difference in trends between the two species is observed among larvae as measured as pupation site preference. Manning and Markow (1981) showed that D. simulans larvae prefer to pupate in lighter places than do D. melanogaster larvae. The authors demonstrated that such light preferences (when compared with light preferences prior to late third instar) were highly specific and restricted to the time just before pupation.

2.2 - Methods

2.2.1 - General pattern of selection and environmental conditions

Directional selection for pupation site prefer-

ence along a gradient of light was carried out both for
light and dark preferences. At most generations the ovi-
position site preference of the females issued from the
selected pupae was scored in a choice situation where
single females had to choose between two contrasted light
intensities to lay eggs. Before further describing the
method used to investigate this possibility of a corre-
lation between larval and adult photopreferences, I shall
treat at some length the various light conditions to
which the animals of each selected line were exposed.
These conditions constituted the main difference between
the selection design I used and those previously used by
other workers.

The investigation of the possibility of an en-
vironmentally induced alteration of larval preferences
was made by varying the light conditions to which iden-
tically selected replicates were exposed. Figure 1 shows
the general pattern of selection and environmental con-
ditions which was initially to be followed in each select-
ion experiment. However, the experiments described in this
chapter did not conform as exactly to this pattern as did
all the subsequent experiments (chapters 3 and 4) but the
principle was identical.

To summarize, replicated lines were selected
both for light or dark third instar larva preferences
and thereafter two types of environmental conditions were

```
preference
behaviour                                              E

                |
                |
                |            ┌"logical continuity"    ——— reward
                |            |
  high          |           /
                |     2 S.L./
                |         \
                |          └"logical discontinuity" ——— trauma
                |
                |          ┌"logical continuity"    ——— reward
                |          |
  low           |     2 S.L.
                |          |
                |          |
                |          └"logical discontinuity" ——— trauma
                |
                |_____
                                          progress of selection
```
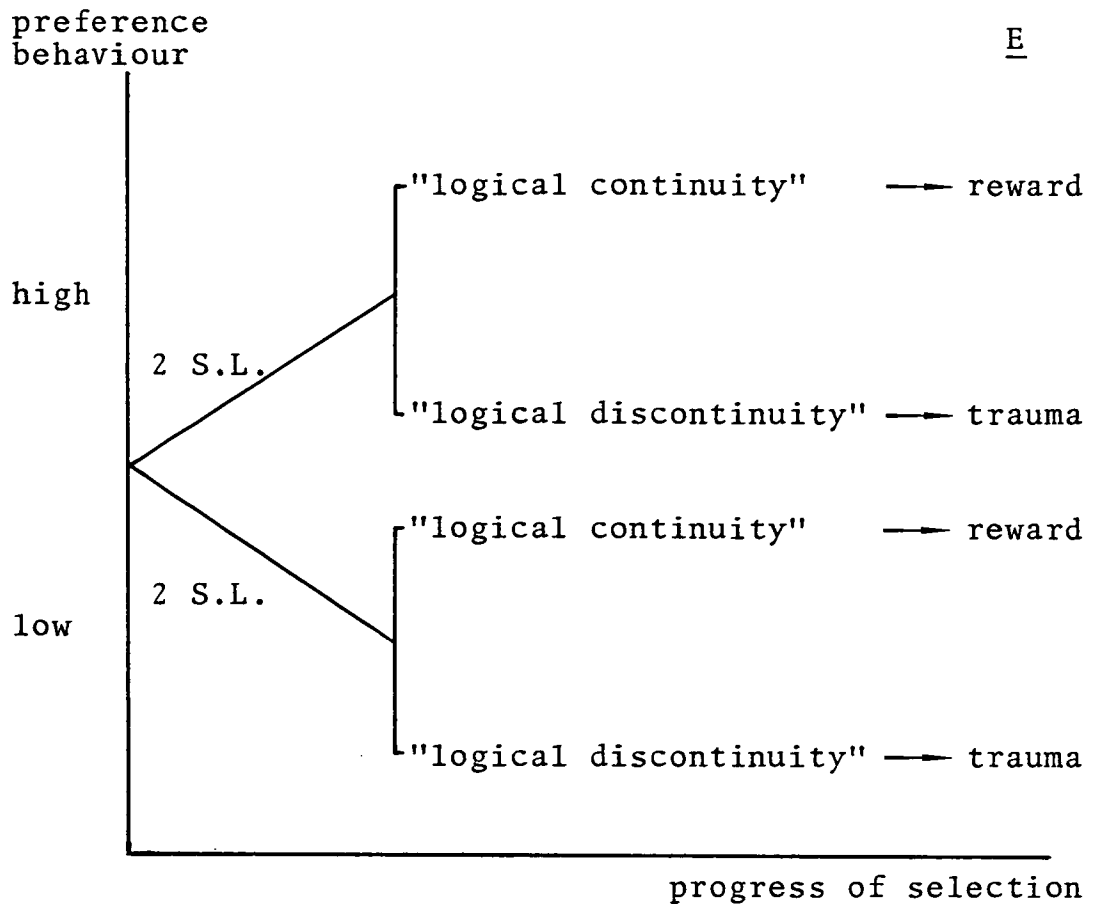
Figure 1. General pattern of selection and environmental
        conditions (E) of the selected lines (S.L.).

faced by early pupal stages, as contrasted as possible
with respect to the choice the late larvae had made. The
first type of environment was characterized by a kind of
"logical continuity" between the preference shown by the
larvae as measured by specific light intensity chosen for
pupation and the later light intensity to which pupae and

adults were exposed. One might regard these first environmental conditions as a type of "reward" which tends to enhance the preference indicated by the choice. The second type of environment offered by contrast a complete "logical discontinuity" compared with the previous situation and one might then regard it as a kind of thwarting situation or "trauma" which tends to contradict the preference indicated by the choice.

2.2.2 - Strain and medium used

The strain of D. simulans used was descendent of eight wild females and four wild males caught at a fruit market in Edinburgh. Approximately one hundred flies of the first progeny from these twelve flies (or more since all the females had already been inseminated when captured) were pooled together with approximately one hundred flies of a recently caught (unlabelled) stock from the Institute of Animal Genetics at Edinburgh. The flies were kept in a population cage for four generations to form the base population. The flies were reared in one-third pint glass milk bottles containing standard medium of the following composition (later referred to as standard medium) :

cornmeal (maize meal)	150 g
treacle	130 g
flaked yeast	22 g
agar technical 3	20 g
nipagin M	1 g

propionic acid   5 cm^3

water   1600 cm^3

All the components except the nipagin were mixed at 20°C
then boiled for two to three minutes. The nipagin was
added when cooling down (at about 60°C). Saccharomyces
cerevisiae in dried form was used when inoculation of
living yeast was necessary.

2.2.3 - Light gradient apparatus used for larval photo-
           preference

A cool white light tube horizontally position-
ed in the incubator ceiling constantly illuminated the
gradients from a distance of 50 cm. An opaque black rect-
angular box (Figure 2) containing 2 cm of standard medium
without living yeast formed the bottom of the gradients.
A set of five holes (Ø 1.5 cm) on both sides of the boxes
allowed a lateral aeration through foam stoppers. Another
set of ten holes (same diameter) also fitted with foam
stoppers allowed an aeration from above. The top of the
boxes was made up of a succession of ten filters absorb-
ing the incident light to provide a regular gradient
(10 % to 100 % absorbed light). The middle of the boxes
allowed the maximum light intensity (irradience of 40
F.C.) to reach the surface of the medium and the two ends
were opaque to light. The lids were fixed to the boxes
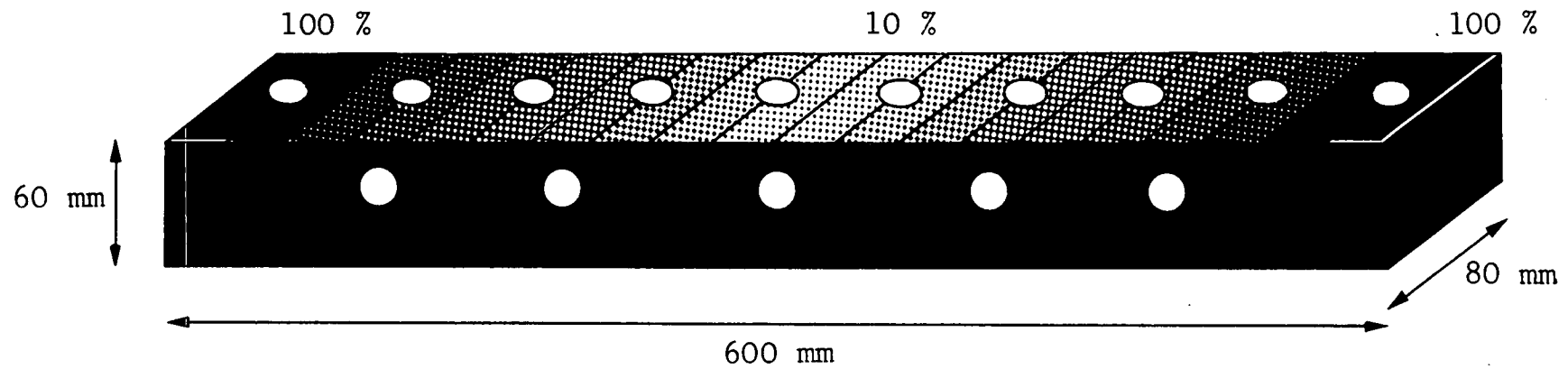
Figure 2. Light gradient apparatus used for larval photopreference in D. simulans.

with black tape. Eggs were removed from the egg laying medium (see under 2.2.4) and counted under a magnifying-glass, although this counting procedure was not very accurate because some eggs were in tight groups. The eggs were deposited in two rows of about seventy-five, respectively at 1/4 and 3/4 of the length in order to avoid any parallel selection for activity (the distance from the eggs to both extremes of light being the same). One hundred and fifty eggs per box was the sample size chosen in order to avoid the effect of larval density, since a relation between pupation site and density has been found at high densities (Sokal et al., 1960). Two boxes were always run simultaneously per selected line (300 eggs per line per generation). All the experiments were performed at 25°C ± 1°C and the level of relative humidity was kept close to saturation in the gradient apparatus.

2.2.4 - Method of egg collection

Experiments were initiated by eighty fertilized females issuing from the base population, being allowed to lay eggs in egg collection pots to form the parental generation (O). In subsequent generations the selected females of all lines were put for nine hours in pre-laying light conditions before their transfer to egg collection pots . Four to five days old fertilized females in culture bottles containing living yeast were therefore

exposed to the same dim light conditions (9 F.C.) for all selected lines. This was done in order to prevent any direct light conditioning of the embryos through their mother's body. At the end of the pre-laying period of nine hours the females were transferred to egg collection pots (∅ 30 mm) containing 2 mm of fresh standard medium without living yeast. These were placed back under the same light (9 F.C.) conditions. After fifteen hours narrow strips of medium about 20 to 30 mm long were sliced out, washed in distilled water and deposited in the gradients where the number of eggs was counted as indicated.

2.2.5 – Recording of pupation site preference (PSP) and selection procedure

One hundred and thirty hours after laying (when the pupae were about one to fifteen hours old) the boxes were opened either in white light for the lines selected for light preference (L-lines) or in deep red light for the lines selected for dark preference (D-lines). This was done in order to insure a continuity with later environmental conditions which corresponded to the "rewarding" situation mentioned earlier. The pupae were then counted in each of the ten areas of lighting conditions and the mean of classified pupae was computed to give a mean pupation site preference ($\overline{PSP}$) in the following way :

$$\overline{PSP} = \frac{\sum\limits_{i=1}^{10} x_i f_i}{n}$$

where x_i = percent of light absorption of ith area,
f_i = number of observations in ith area, and n = total
number of observations. Replicated lines were always run
simultaneously, all with 300 eggs per line per generation.
Flies were always mated in pairs in vials and CO_2 was used
as anaesthetic throughout.

2.2.6 - Control for a "gradient effect"

"Gradient effect" was tested by using two
homogeneously lighted boxes (ten areas of 10 % of absorb-
ed light) as controls for possible effects of the base of
the gradient on larval distribution.

2.2.7 - Control-line (C-line)

An unselected line descended from the base
population was maintained as control to examine PSP of a
line which underwent a degree of inbreeding similar to
that of the selected lines. For this purpose twenty pairs
picked and mated at random were used for each generation.
The flies of this line were exposed to a 12 hour light

(49 F.C.)/12 hour dark cycle. Owing to time problems this line was only kept until generation five of selection in this first experiment.

2.2.8 - Light preference rewarded lines (L-lines)

From the two boxes run simultaneously in each selected line forty-two to forty-six pupae were removed with a paintbrush and put in pairs in vials to guarantee about twenty flies of each sex. At eclosion about twenty couples were then put all in fresh vials to be used as parents of the next generation after random mating. Since there were occasionally not enough pupae in the areas where 10 % of the incident light was absorbed a few pupae sometimes had to be taken from the areas where 20 % of the light was absorbed (and exceptionally 30 %). The vials were provided with a drop of a suspension of living yeast and four to five days after emergence the males were re- moved and the females transferred to pre-laying conditions (see under 2.2.4.). Selected pupae and adults were kept under a 12 hour light (49 F.C.)/12 hour dark cycle. Two replicated L-lines (L1 and L2) were always treated simul- taneously, the first for twenty-eight and the second for twenty-three generations of selection.

2.2.9 - Light preference traumatic line (LT-line)

At generation twenty-two of selection an additional line selected for light PSP was set up to undergo a traumatic environmental treatment. This LT-line originated from twenty-one pairs eclosing from forty-four L1- and L2-line pupae, all removed from the areas where 10 % of the incident light was absorbed. Selected pupae and adults of this line were treated as those of L-lines for five generations except that they were exposed to a 12 hour dim light (9 F.C.)/12 hour dark cycle. L1-line and LT-line were always run simultaneously.

2.2.10 - Dark preference rewarded lines (D-lines)

Forty-four pupae were always selected from the areas where 100 % of the incident light was absorbed. Selected pupae and adults were kept under a 12 hour dim light (9 F.C.)/12 hour dark cycle. Two replicated D-lines (D1- and D2-lines) were always run from twenty parental pairs and treated simultaneously, the first for twenty-three and the second for twenty-four generations of selection. Dim light was used (as for LT-line) in order to insure that some light could allow the flies to mate since complete darkness inhibits mating in D. simulans (see under 2.1.1).

2.2.11 - Dark preference traumatic lines (DT-lines)

At the fifth generation of selection an additional line selected for dark PSP was set up to undergo a traumatic environmental treatment. This DT1-line originated from twenty parental pairs of flies coming from sixty-five D2-line pupae, all removed from the area where 100 % of the incident light was absorbed.

At the eight generation another similar additional line (DT2-line) was set up for the same purpose from nineteen parental pairs coming from fifty-two DT1-line pupae, all removed from the areas where 100 % of the incident light was absorbed. Selected pupae and adults of these lines were treated as those of D-lines except that they were exposed (immediately after their removal from the boxes) to a constant horizontal white light source of an irradience of 236 F.C..DT1-line was treated this way for twenty-five generations and DT2-line for fifteen generations. D-lines and DT-lines were always run simultaneously.

At generation 16 selection was relaxed for one generation in all L-, D- and DT-lines.

2.2.12 - SubD1-line and subDT1-line maintained under uniform lighting conditions

Sub-samples of both D1-line and DT1-line were

simultaneously cultured from generation eighteen to generation twenty-two under identical conditions of light and without further selection. Forty residual pupae were removed for this purpose from the dark ends of the boxes of both D1- and DT1-lines. The boxes were opened in white light and selected pupae and adults were all exposed to a 12 hour reduced light (20 F.C.)/12 hour dark cycle. At each generation two sets of one hundred and fifty eggs were run respectively in two boxes for both lines (as in the selection experiments) to record the PSPs of the two lines. The flies were always randomly mated in pairs in vials and twenty inseminated females were allowed to lay eggs in egg collection pots when four or five days old. SubD1- and subDT1 - lines were always run simultaneously in the gradient apparatus.

2.2.13   - <u>SubDT1-line without further traumatic treatment (DTD-line)</u>

At generation 22 of selection, forty residual pupae of DT1-line were removed from the dark ends of the gradients to set up a DTD-line which had no more traumatic treatment, but was instead treated like D-lines. Pupae and adults selected for dark PSP were thus exposed to a 12 hour dim light (9 F.C.)/12 hour dark cycle and PSP was always recorded simultaneously to that of DT1-line for seven generations.

2.2.14 - Lines kept in permanent light and permanent darkness for twenty-nine generations (PL- and PD-lines)

Two samples of fifty females each coming from the base population initiated respectively one PL-line kept in permanent light (constant irradience of 90 F.C.) and another PD-line kept in permanent darkness. These flies were not artificially selected and were kept for twenty-nine generations in bottles where no random mating was imposed. Every fifteen days twenty to thirty females were transferred for twenty-four hours into one-third pint milk bottles with fresh medium and a few drops of living yeast suspension. This transfer was performed under white light for the first line and under deep red light for the second line. At generations 14, 19 and 29 twenty fertilized females (five to seven days old) were allowed to lay eggs in egg collection pots in reduced light conditions (20 F.C.) and PSPs of both lines were recorded in the gradient apparatus.

2.2.15 - Reciprocal hybridizations between selected lines

Several reciprocal hybridizations between selected lines were carried out by crossing twenty females with twenty males in single pairs in vials. These flies were always eclosed from pupae previously put singly

in vials. Fertilized females were always placed in pre-
laying conditions for nine hours before their transfer to
egg collection pots.

2.2.16 - <u>Statistics used to compare larval photo-
preferences between lines</u>

Since larvae showed a slight tendency to
aggregate near the lateral plugs of the gradient apparatus
to pupate,the distribution of the pupae along the gradient
was often not a normal one. In order to compare such
distributions the median test was then used and probabi-
lity values were determined by a chi-squared table.

2.2.17 - <u>Recording of oviposition site preference (OSP)</u>

At the end of the fifteen hours'period of egg
collection,fifteen to twenty selected females (five to six
days old) of L-, D- and DT-lines were placed individually
in vials to test their photopreference at the time of ovi-
positing. Several authors have reported that <u>Drosophila</u>
females tend to oviposit near sites previously used for
oviposition by other females (in Del Solar, 1968), there-
fore it was necessary to test females individually. As
shown in Figure 3 the test vials contained 2 cm of stand-
ard medium without living yeast and a thin vertical
plastic separation (opaque to light) was inserted into

75 mm

foam
stopper

light
source

opaque
separation

standard
medium

25 mm
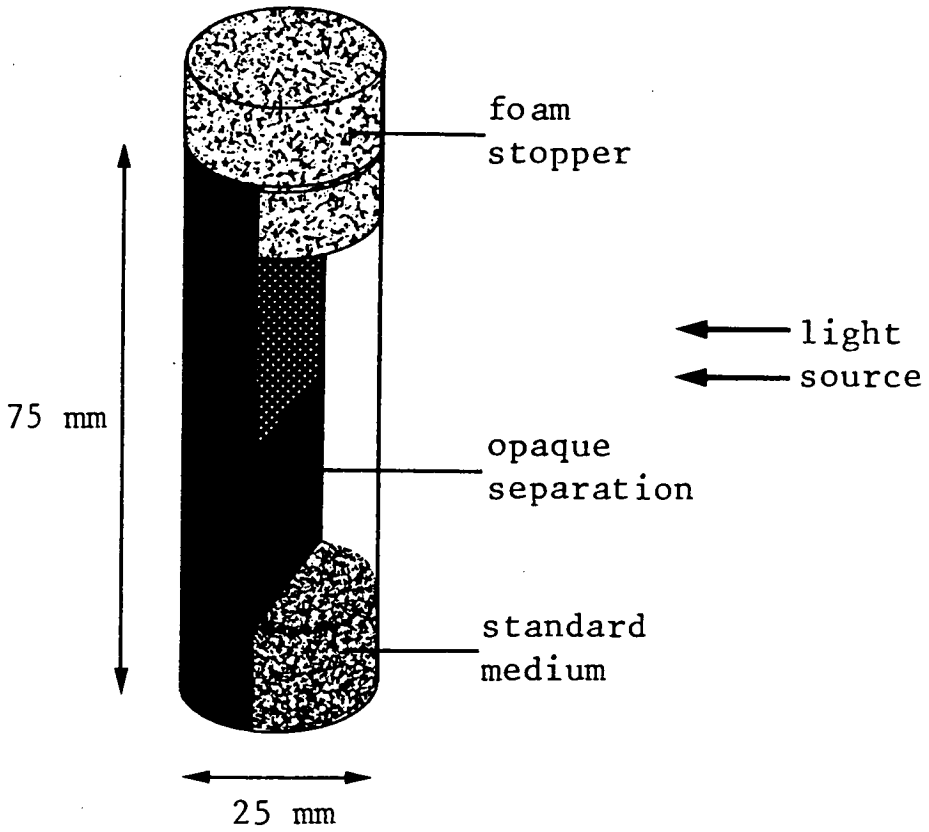
Figure 3. Test vials used for
oviposition site preference.

the medium along the diameter. One half of the vials was
illuminated by a direct horizontal cool white light
(source situated at 30 cm) of an irradience of 213 F.C. at
the surface of the vial. The other half of the vials was
lined with black paper from the bottom to the top. Females
to be tested were anaesthetized as lightly as possible in
order to facilitate their transfer into the test vials.
These were then kept horizontally for ten minutes in
reduced light (20 F.C.) before being exposed to the test
light conditions. Twenty-four hours later the eggs laid
in each half of the vials were counted under a binocular.

Although the flies of all lines to be tested were
thus exposed for twenty-four hours to the same light
intensity of 9 F.C. (including the pre-laying light
conditions period),we shall consider under 2.4.1 the
possibility that the test procedure did not take into
account an effect due to the differential conditioning
of the flies which had been previously raised either in
almost darkness (such as D-lines) or in more light (such
as L- and DT-lines).

2.2.18 - <u>Statistics used to compare adult photo-
preferences</u>

Square roots of the percentages of eggs laid
were first arcsine transformed before 95 % confidence

limits were plotted with the means of the transformed data. The above angular transformation was appropriate to the percentages measured since it stretches out both tails of a distribution of proportions and compresses the middle.

2.2.19 - Fecundity of the selected flies

The fecundity of the selected females was estimated in L-, D- and DT-lines from the scores of the oviposition site preference, by adding the number of eggs laid per female per twenty-four hours in both light and dark. As pointed out under 2.2.17 this procedure might actually not have only measured true fecundity but also some inclination to lay eggs under the test conditions, since these might have represented a stronger contrast of lighting conditions for D-lines flies than for the other lines investigated.

2.2.20 - Egg-larval mortality

An estimation of egg-larval mortality rates was obtained at most generations from the difference between the number of pupae found in each box and the number of eggs which had been deposited. Because of the lack of precision of the counting procedure mentioned under 2.2.3, I based the estimate on an initial number of 150 $\pm$ 20 eggs in all boxes.

2.2.21 - <u>Mortality at the pupal stage and sex ratio of the flies emerging from the selected pupae</u>

An estimation of pupal mortality rates was obtained at most generations from the number of flies emerging from a sample of forty pupae retained for selection. The relative proportion of males and females among the emerging flies was used as an index of the sex ratio of the adults emerging from the selected pupae.

2.3 - <u>Results</u>

2.3.1 - <u>Selection for light PSP (L1- and L2-lines)</u>

Figure 4 shows that both replicates of L-lines did not respond to selection. The rather marked fluctuations recorded at generations 7 and 9 for both lines are commonplace in selection experiments and might have been caused by some abrupt variation of an environmental parameter, such as medium contamination, atmospheric pressure or incubator humidity level. Similar observations will be reported later on for other lines run simultaneously. Table 1 (p. 91) shows that the cumulated mean responses to selection were low after ten generations of selection (7.5 for L1-line and 11.5 for L2-line). The relative stability of later scores of PSPs of both L1- and L2-lines suggests that the character successfully selected in the
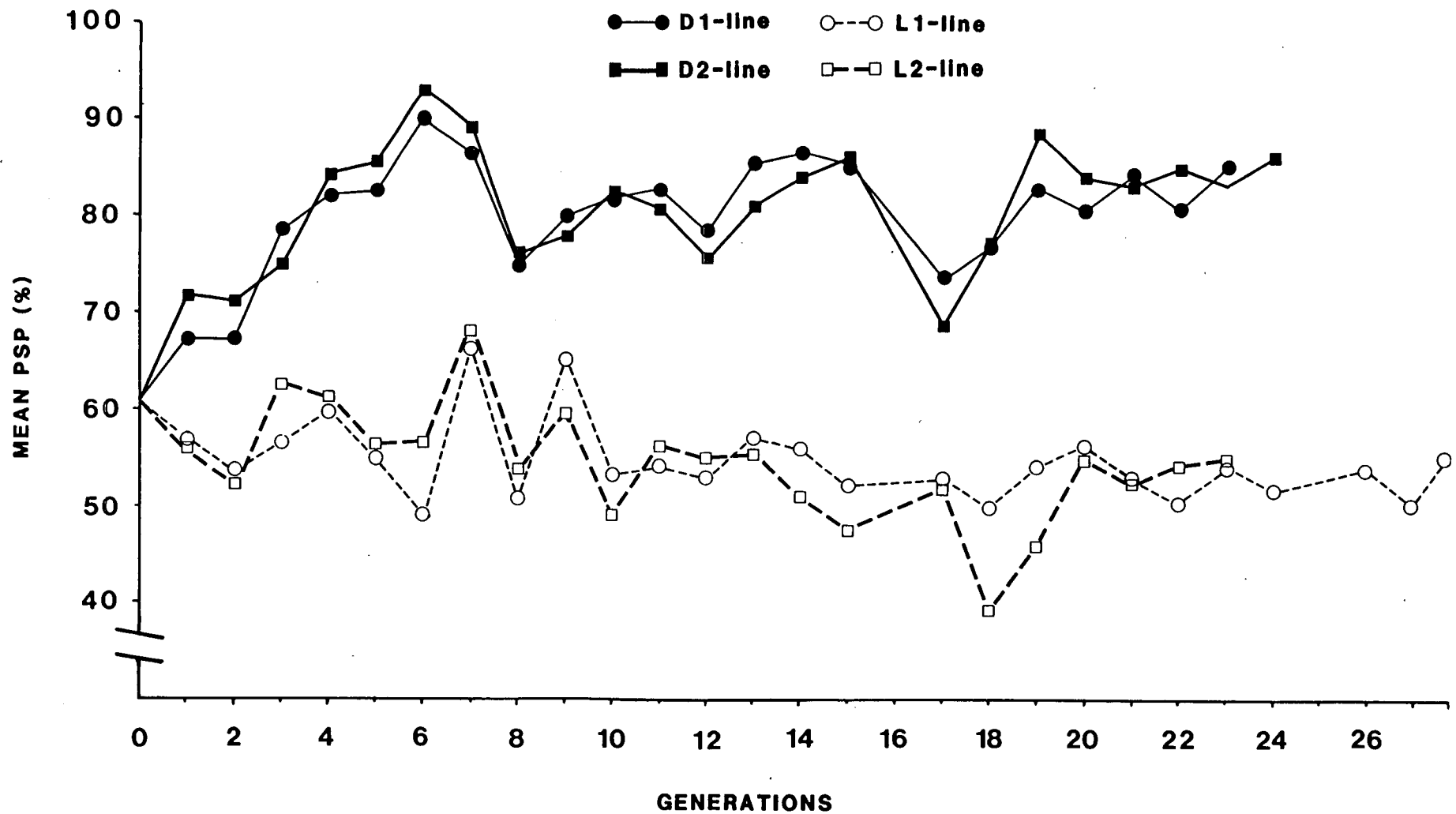
Figure 4. Mean pupation site preference of L1-, L2-, D1- and D2-lines plotted against generations of selection.

upward direction in D-lines was apparently not strongly subject to inbreeding depression. For the first ten generations of selection the average response to selection (R) per generation was given by the slope of a regression line fitted to the generation means of preferences (Falconer, 1981) and yields values of R = 0.054 for the L1-line and R = 0.300 for the L2-line. Both correlation coefficients between R and progress of selection (r = 0.033 for L1-line and r = 0.188 for L2-line) are not significant when tested against ρ = 0 (correlation = 0).

The realized heritability h^2 was estimated from the ratio $\frac{R}{S}$ (Falconer, 1981) where S is the selection differential measuring the average superiority of the selected parents. For the first ten generations of selection, the generation means of preferences were therefore plotted against the cumulated selection differentials. The average value of the ratio $\frac{R}{S}$ was then given by the slope of the regression line fitted to the points. The realized heritabilities had low values of h^2 = 0.001 for L1-line and h^2 = 0.005 for L2-line. Both are not significant when tested against β = 0 (slope = 0).

Since some selected pupae were occasionally removed from areas of the gradient where more than 10 % of the incident light was absorbed (see under 2.2.8) this was taken into account by computing adjusted selection differentials as reported in table 1.

The PSPs of L1-line and L2-line remained close to each other throughout the experiment and phenotypic variances remained at about the same level as selection progressed. Intra-line variation of PSP was low and table 2 shows that nine cases (of pairs of replicate box distributions compared) out of forty-five were significantly different at the .05 level. Inter-replicate lines' variation of PSP (between L1- and L2-lines) was apparently slightly greater which could have partly been due (besides a drift effect) to the small differences between selection differentials applied between the lines (see table 1). Table 3 shows that fourteen cases out of forty-eight were significantly different at the .05 level. This table gives four probability values per generation because there were two boxes per line per generation (see under 2.2.3) which makes four combinations of comparison of pairs of box distributions. Such combinations will always be presented in the same order in tables, with the sign (') referring to the first box of a pair and the sign (") to the second box of the same pair.

2.3.2 - <u>Selection for light PSP with traumatic environmental treatment (LT-line)</u>

Figure 5 shows that PSPs of LT- and L1-lines remained close to each other and this experiment was stopped after five generations of LT-line because the
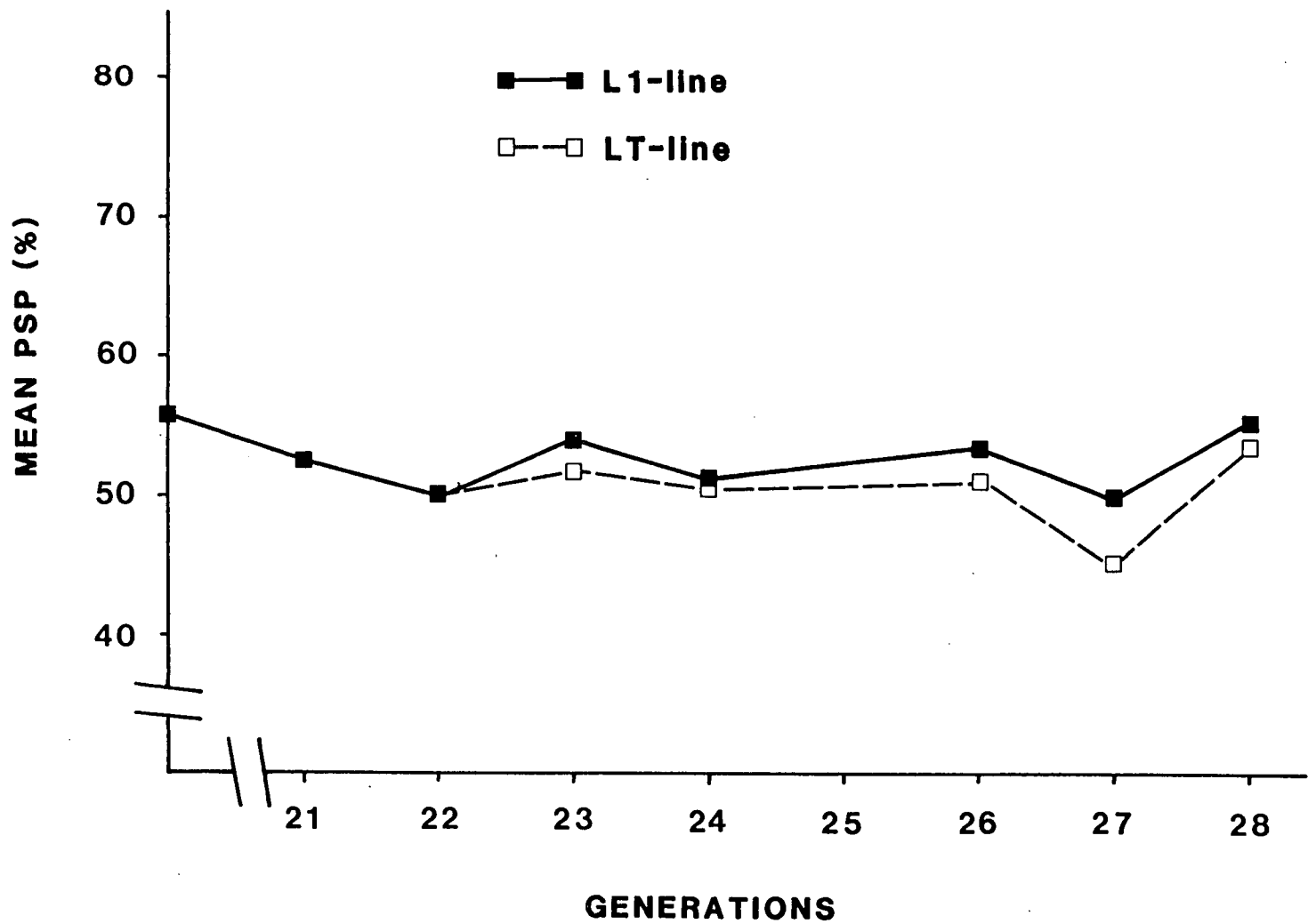
Figure 5. Mean pupation site preference of L1- and LT-lines plotted against generations of selection.

preferences were getting more and more similar as the treatment progressed. Table 4 shows that after three generations of LT-line treatment (twenty-second to twenty-fourth) the PSPs of the two lines were very similar indeed with most probability values falling between <.5 and <.9. Selection was relaxed at generation 25.

2.3.3 - <u>Selection for dark PSP (D1- and D2-lines)</u>

Figure 4 shows that both replicates of D-lines did respond to selection and a plateau was already reached by generation six. This result could have been predicted considering the relatively small size of the sample used to set up the base population. Table 5 shows that after six generations the cumulated mean responses to selection were high (29.4 for D1-line and 32.3 for D2-line) compared with those of L-lines (11.5 and 4.0, see table 1), after the same number of generations. For the first seven generations the average response thus had high value of R = 4.088 for D1-line and R = 4.186 for D2-line. The realized heritability of D1-line was 0.177 and that of D2-line was 0.183. When tested against $\beta = 0$ the former is significantly different at the .001 level and the second at the .01 level.

It is worth noticing that by generation 7 the selection differentials were very low and as a result

the responses to selection were necessarily weakened.

At generation 7 the flies were mass mated but still selected which apparently caused a decline in the responses. Interestingly such homeostasis might have largely operated through sexual selection if the dropping of the mean preference which occurred in both D1- and D2-lines was not just due to an uncontrolled environmental variation. The selection differentials were consequently higher again and the responses calculated from generations 8 to 15 were R = 1.319 for the D1-line and R = 1.048 for the D2-line. The realized heritabilities calculated for these generations were h^2 = 0.058 for D1 and h^2 = 0.048 for D2. When tested against β = 0 the first has p $\sim$ .05 and the second p < .2 to be significantly different. This suggests that although an apparent plateau was reached after six generations of selection, some lesser selection was still possible afterwards, at least in the D1-line.

At generation 16 no selection was applied and the flies were again mass mated. Again this might have caused a substantial decline in both D1- and D2-lines responses (unless this decline was due to random fluctuations) but further selection appears to have reestablished the previous level of the preference values in about two generations.

Intra-line variations of PSPs of both D1- and D2-lines were low and table 6 shows that only six cases out of forty-five were significantly different at the .05 level. The same can be said about inter-replicate lines' variation of PSP measured between D1 and D2 and table 7 shows that between generations 1 and 12 of selection five cases out of forty-eight were different at the .05 level.

The standard errors on the mean preferences have been calculated at each generation but they are not reported here since they were not really adequate when distribution patterns differed slightly from normal (see under 2.2.16). Nevertheless, these remained at about the same level as selection progressed as has been often observed in selection experiments (Falconer, 1981), although the expected loss of genetic variance might be expected to lead to a progressive decline of the observed phenotypic variance. Falconer suggests several possible reasons for this phenomenon and I shall come back to this point at the end of this chapter.

Tables 8 to 11 show that D-lines separated very quickly from L-lines. The difference observed for the first two generations is perhaps slightly overestimated because of the rather low values of PSP of both L-lines at the outset when compared with later scores.

2.3.4 - <u>Selection for dark PSP with traumatic environ-
mental treatment (DT-lines)</u>

Compared with D-lines, the two replicated DT-
lines showed more intra- and inter-lines' variation and
the shape drawn by the lines of PSP (figure 6) was more
erratic. Intra-line variations of PSPs of both DT1- and
DT2-lines are given in table 12 and between generations
6 and 24 of selection eight cases out of thirty-two were
different at the .05 level.

Table 13 shows inter-replicate lines' variation
of PSP and between generations 14 and 21 six cases out of
twenty-eight were different at the .05 level. Interesting-
ly some of the above discrepancies were synchronous in D-
and DT-lines. Since all the four lines were always run
simultaneously, these coincident variations are likely to
have a common environmental cause as suggested earlier for
the L-lines (see under 2.3.1). A comparative look at both
tables 6 and 12 suggests that such disturbances might have
occurred at generations 10, 13, 17 and 19.

Tables 14 to 17 show that apparently the trau-
matic treatment significantly altered the mean PSP of DT-
lines (compared with D-lines) after two or three gener-
ations. After about five to six generations of repeated
exposure to light, the mean PSPs of both DT-lines became
significantly different from the mean PSPs of both D-lines
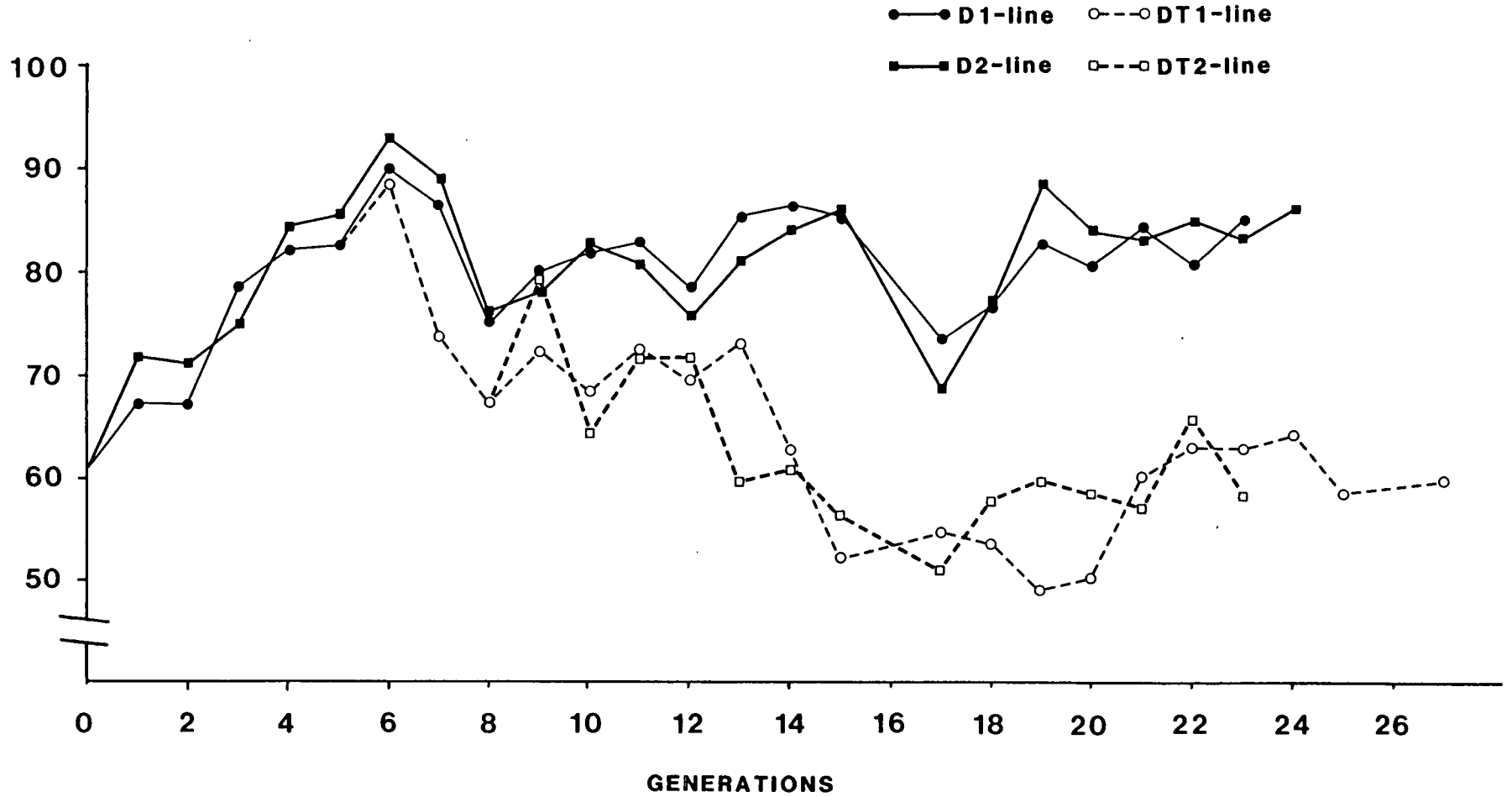
Figure 6. Mean pupation site preferences of D- and DT-lines plotted against generations of selection.

in most cases at the .001 level. Between generations 14
and 24 the comparison of all possible pairs of boxes
(tables 14 to 17) shows that one hundred and forty-three
cases out of a total of one hundred and forty-eight were
different at the .05 level.

For the sake of comparison, table 18 shows the
cumulated mean "responses" of DT-lines as measured as a
trend towards more light PSPs. These cumulated "respon-
ses" rapidly reached high values, even though the select-
ion differentials (of selection for dark PSP) were marked-
ly increased. The responses measured simultaneously in D-
lines were thus obtained with lower selection different-
ials (13.5 for D1 and 15.9 for D2 at generation 15). The
apparent "responses" to the traumatic treatment measured
this way were then R = -2.309 for DT1 and R = -2.619 for
DT2 between generations 6 and 15 of selection. When test-
ed against $\beta = 0$ the former is significant at the .02
level and the latter at the .005 level. If the above
series of generations is divided into two subseries
(generations 6 to 10 and 11 to 15) the "half-responses"
measured this way become R_1 = -4.150, R_2 = -4.770 for
DT1 and R_1 = -4.210, R_2 = -4.150 for DT2. None of these
"half-responses" are significantly different when tested
against $\beta = 0$ (p < .2 for DT1 and p < .3 and <.1 for DT2),
but the number of generations considered is small.

These results might still suggest that the separation of DT-lines from D-lines was progressive or at least delayed,although the slopes of the "responses" measured between successive generations (table 18) do not support any regular progressive pattern.

Figure 6 shows that after about ten generations of traumatic treatment both DT-lines tended to have slightly darker PSP. In addition,data which are not reported here because high mortality made them unreliable further suggested that between generations 28 and 31 the PSP of DT1-line were perhaps becoming even closer to those of D-lines.

2.3.5 - SubD1- and subDT1-lines maintained under uniform light conditions

Figure 7 shows that subD1- and subDT1-lines remained separated when cultured under identical light conditions. Regrettably this experiment was only carried on for four generations but all pairs of box distributions compared between generations 19 and 22 were significantly different at the .001 level (table 19). According to what has been observed about relaxing selection in D-lines (see under 2.3.3) one would have expected subD1-line PSP to go back towards more light PSP through homeostasis, but this was not observed. Nonetheless,it must be remem-
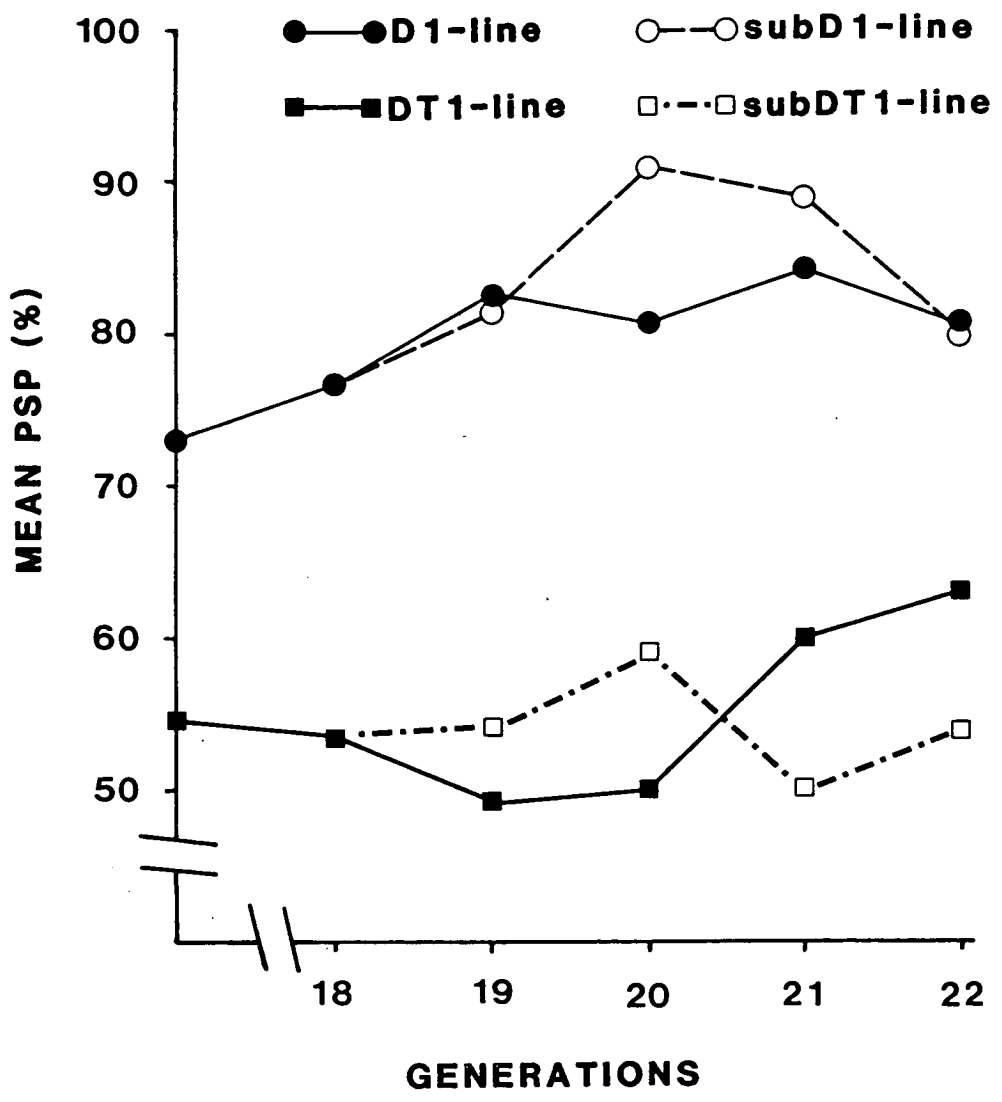
Figure 7. Mean pupation site preferences of D1-, DT1-,
subD1- and subDT1-lines plotted against
generations of selection.

bered that the flies used as parents in subD1-line were always mated in pairs since homeostasis was suspected (see under 2.3.3) to be very effective when operating through sexual selection. These results at least suggest that DT-line flies were genotypically different from D-line flies.

2.3.6 - SubDT1-line without further traumatic treatment (DTD-line)

Figure 8 shows that DTD-line tended to have more dark PSP when treated again like ordinary D-lines. Despite the significant differences in PSP shown in table 20 more data are obviously needed here as this experiment was done with highly inbred animals. Selection was re-laxed at generation 26 and flies were still mated in pairs but subsequent recording of PSP cannot be directly com-pared with D-lines PSPs since these were no longer se-lected after generations 23 and 24. When looking at the apparent shift of DTD-line toward darker PSP it is worth considering that DT1-line had shown a similar trend from generation 19 onwards.

2.3.7 - Lines kept in permanent light (PL-lines) and permanent darkness (PD-lines) for twenty-nine generations

Table 21 shows that PSPs of lines kept either in
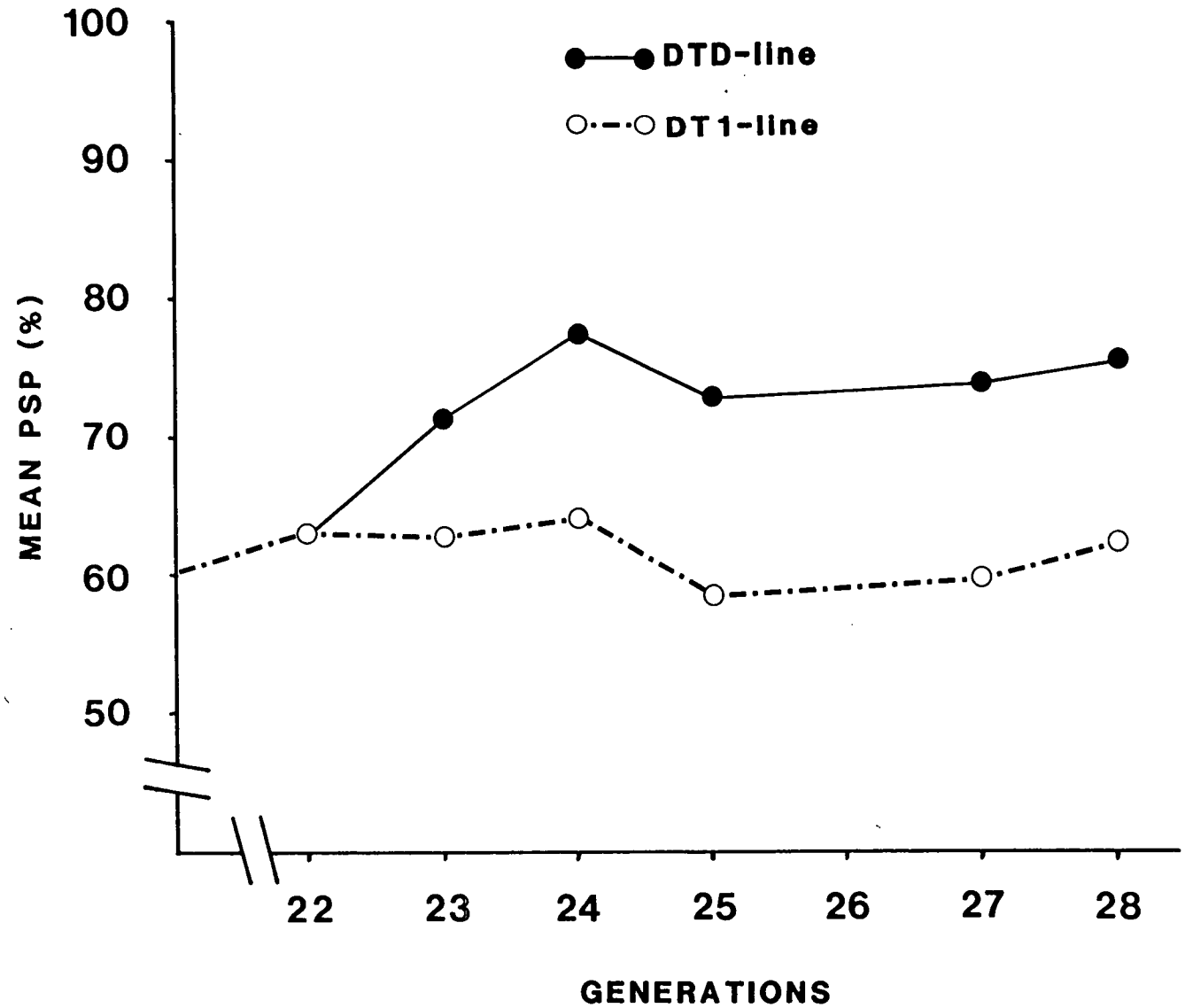
Figure 8. Mean pupation site preference of DTD- and DT1-lines plotted against generations of selection.

permanent light or in permanent darkness seemed to remain similar and stable in both lines. As we shall see later these data provide only limited information relevant to the observed discrepancy between D-lines and DT-lines, although they were initially collected for this purpose.

2.3.8 - Control-line (C-line)

As mentioned, the PSPs of the control-line were only recorded at generations 3 and 5 in which four boxes were run instead of two for more precision. Table 22 shows that mean PSPs at generations 3 and 5 were significantly different at the .05 level in only two cases out of sixteen. Since these results did not rule out a possible longer term change in PSP attributable to inbreeding depression a more tight control was carried out in further experiments (see chapter 3).

2.3.9 - Control for a "gradient apparatus effect"

The distribution of pupae in two boxes run with homogeneous incident lighting conditions showed no significant difference from a homogeneous distribution (table 23). There was still a weak tendency (already mentioned) for third instar larvae to select pupation sites near the lateral aeration plugs as well as at both ends of the boxes where presumably they found slightly dimmer light than elsewhere.

2.3.10 - L2-line X D2-line reciprocal hybridizations

At generations 21 and 22 of selection reciprocal hybridizations were carried out between the L2- and D2-lines and the summarized results are presented in table 24. Probability values of tables 25 and 26 support a strong involvement of the X chromosome in larval photo-preference since PSPs of progeny from the reciprocal hybrids were different and more similar to those of the female parent . Six cases out of eight are significantly different at the .02 level and all the four distributions compared at generation 22 are different at the .001 level. In table 24 differences significant at the .05 level between progeny PSPs are indicated by an asterisk and the emerging picture is entirely compatible with an X-linked dominant genetic basis controlling light PSP, unless a strong maternal effect can account for the results.

2.3.11 - Sex ratios of light and dark preferring progeny issued from L2-line females X D2-line males cross

According to the genetic basis just suggested by the previous results one would expect no sexual di-morphism in PSP of progeny from L2-line females X D2-line males cross. This is because among such progeny both sexes are equally likely to get one X chromosome carrying

the dominant gene(s) determining light PSP. Fifty pupae
issued from this cross were thus removed from extreme
light conditions in the boxes and sixty pupae from ex-
treme dark conditions of the same boxes. Proportions of
males (respectively 46.8 % and 53.7 %) found in these
samples did not show any significant departure from a 1:1
ratio. Comparisons of percentages observed with 50 % give
p ∿.66 in extreme light and p ∿.59 in extreme dark. Re-
grettably sex ratios of progeny issued from the reciproc-
al cross were not recorded.

2.3.12 - D2-line X DT1-line reciprocal hybridizations

Results given under 2.3.5 suggested a genetic
difference between DT-line flies and D-line flies there-
fore it was worth attempting to hybridize these lines as
well. Table 27 summarizes PSPs of progeny issued from the
reciprocal hybridizations between D2-line and DT1-line.
Progeny from the reciprocal hybridizations appear to
have different PSP,although a mere maternal effect cannot
be excluded here. Table 28 shows that PSP of the progeny
from DT1-line females X D2-line males differed markedly
from D2-line but not from DT1-line. Since the situation
was different for progeny from D2-line females X DT1-line
males the emerging picture looks as if DT-line genotype
may have influenced larval phototaxis in a similar way to
L-line genotype despite the fact that flies had been se-

lected for twenty-two generations for extreme dark PSP.
Caution is necessary in accepting this interpretation
since only one set of crosses were made and the differ-
ence between D2 X D2 and D2 females X DT1 males progeny
is not significant, as would be expected if X-linked
dominant genes were present in the DT1-line. These re-
sults must also be cautiously treated since a fairly
high larval mortality was observed. This rather odd
observation will be compared in chapter 3 with similar
observations obtained with D. melanogaster. About 85 %
of the descendants from DT1-line female X D2-line male
cross reached the pupal stage but for D2-line female X
DT1-line male cross only about 40 % of the descendants
reached the pupal stage. One therefore should not con-
clude that the trends observed in phenotype frequencies
necessarily reflected similar trends in genotype fre-
quencies. Because there was a possibility that some
extra selection for X-linked dominant genes controlling
light PSP had taken place within DT-lines through the
traumatic treatment both the fecundity and mortality
rates must be compared between D- and DT-lines.

2.3.13 - Fecundity of L-, D- and DT-flies

Figure 9 shows that the fecundity of the flies
of both L-lines tended to decrease throughout the select-
ion experiment. The correlation coefficients between fe-
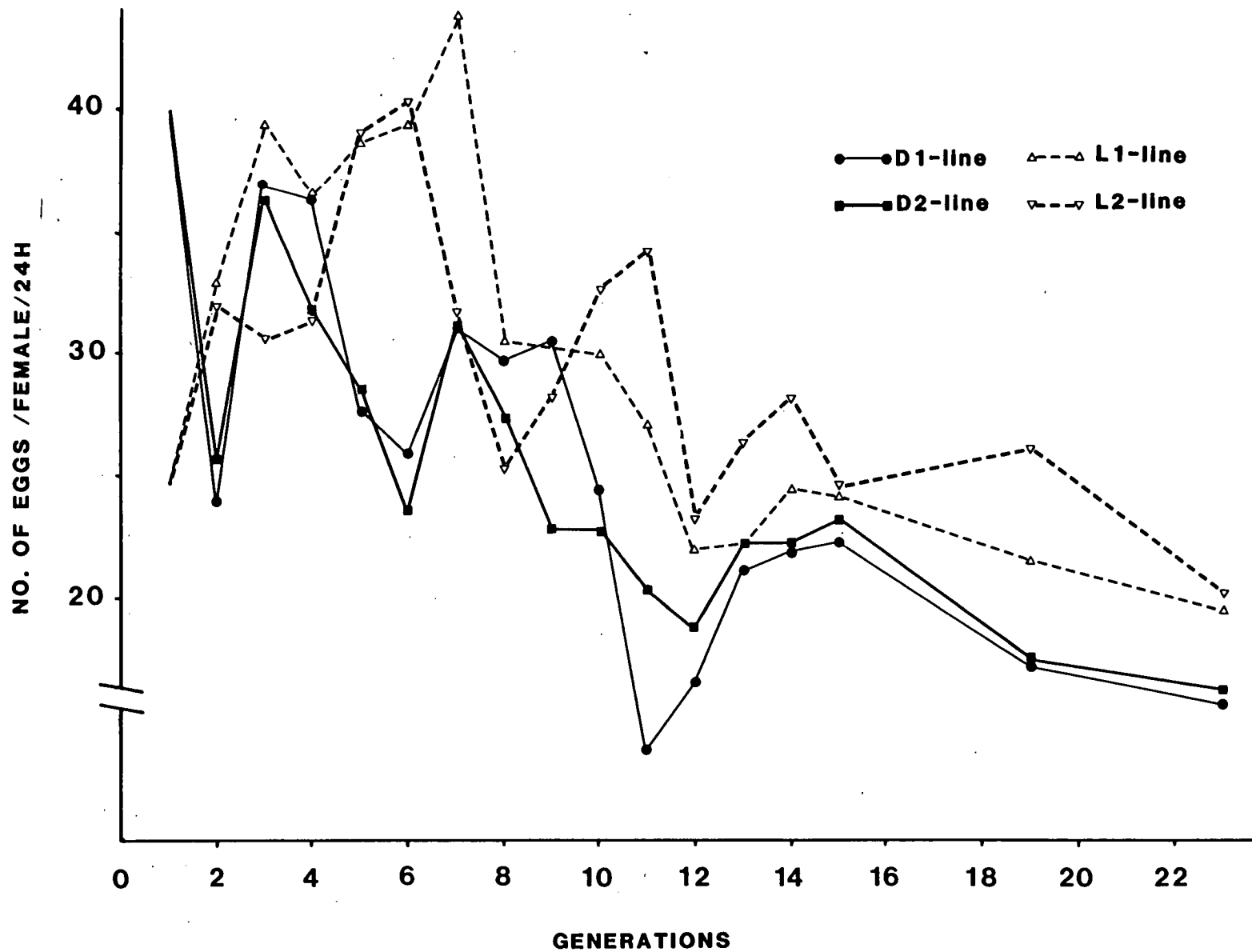
Figure 9. Fecundity of flies of L- and D-lines plotted against generations of selection.

cundity and progress of selection are r = -0.838 for the L1-line and r = -0.683 for the L2-line. Both are significant at the .01 level when tested against ρ = 0 (correlation = 0). The fecundity of the flies of both D-lines declined in a similar way, although at almost all generations D-line scores were slightly lower than those of L-lines as figure 9 shows. Between generations 4 and 10 D-line females were most of the time significantly less fecund than L-line females as indicated in table 29. The correlation coefficient between D1-line fecundity and progress of selection is r = -0.733 and that of D2-line is r = -0.802. Both are significant at the .01 level, when tested against ρ = 0.

The fecundity of the flies of D-lines and DT-lines was compared between generations 10 and 14 (corresponding to the period during which DT-lines significantly diverged in pupation site preference from D-lines) and the results are presented in table 30. Only four cases out of twenty were significantly different at the .05 level but this does not rule out the possibility that some females (having in common genes influencing positive larval phototaxis) were actually laying more eggs within DT-line.

Nevertheless, by looking at the standard deviations of the above means (table 31) it appears that the measure of dispersion around the means is very si-

milar in D-lines and DT-lines. At first sight the trau-
matic treatment did not abruptly diminish the fecundity
of some particular DT-line females, although the definite
absence of differential fecundity rates was not proven.

2.3.14 - Egg-larval mortality

Table 32 shows the approximate mean egg-larval
mortalities of all L-, D- and DT-lines. It is possible
that the "natural" egg-larval mortality was sometimes
slightly overestimated because a few eggs may have been
damaged during the slicing out of the strips of medium
containing them. Nevertheless, the mean egg-larval mortal-
ities varied between 4.7 % and 10.2 % which is reasonably
low.

2.3.15 - Mortality at the pupal stage

Here too the removal of newly formed pupae with
a wet paintbrush may have accidentally hurt some of them
and affected their survival. Therefore the rates of mortal-
ity at the pupal stage reported in table 33 may also have
been occasionally overestimated. From both tables 32 and
33 it seems unlikely that the total preimaginal mortality
(egg-larval-pupal mortality) substantially exceeded 13.3 %.
Although this roughly corresponds to the proportion of
flies selected in all lines at each generation, the dif-

ferences between lines were comparatively small. From eggs to pupae the animals of all lines were always exposed to identical environmental conditions,therefore if selective deaths were significantly altering some gene frequency in one line this should have occurred primarily through differential mortality rates at the pupal stage.

2.3.16 - Sex ratios of L-, D- and DT-flies emerging from selected pupae

The sex ratios of the adult flies emerging from selected pupae were recorded at most generations in L-, D- and DT-lines. Table 34 shows that in none of the lines did the sex ratio differ significantly from 50 %. In this table a comparison of the percentages of males of both D- and DT-lines may seem worth more attention but table 35 shows that the difference was not significant.

2.3.17 - Oviposition site preference (OSP)

As already mentioned the possibility of a correlation between preadult and adult behaviour was studied through oviposition site preference (OSP),and table 36 shows that L-line females did not show significant variation for this behaviour as selection for PSP progressed. As expected by looking at figure 10 no correlation was found for either L-line between OSP and
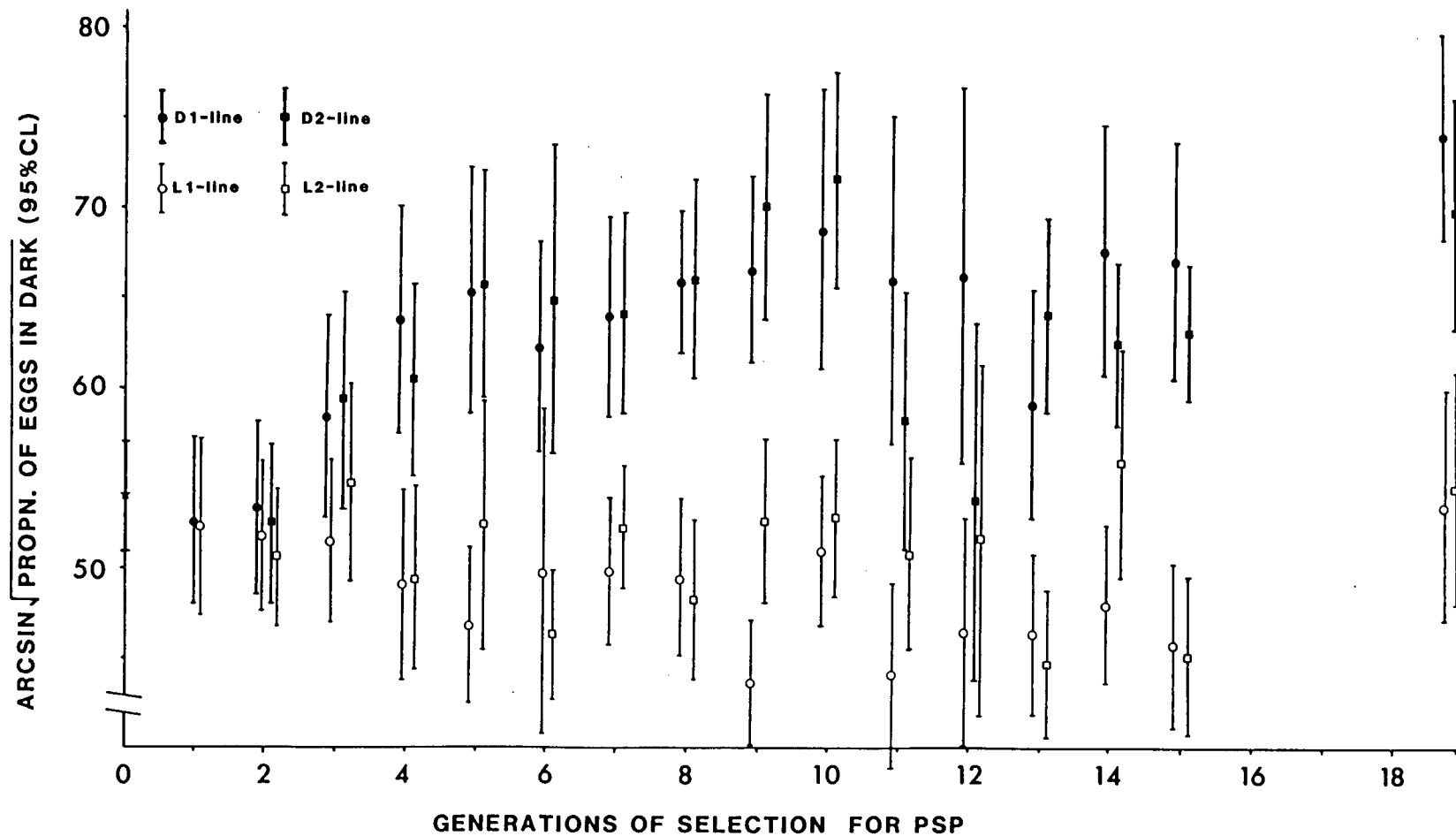
Figure 10. Oviposition site preferences (angular transformations of proportion of eggs laid in dark per female per 24 hours with 95 % confidence limits) of L- and D-lines plotted against generations of selection for pupation site preferences.

progress of selection for PSP from generations 1 to 10. For L1-line r = 0.523, for L2-line r = -0.014 and both are not significant when tested against ρ = 0. This absence of response thus parallels what was observed for PSP in these lines.

By contrast D-line females tended to lay significantly more eggs in dark as selection for PSP progressed (table 36). Both lines diverged rapidly during the first generations of selection and figure 10 shows that the 95 % confidence limits of D-line means overlap only once those of L-lines between generations 4 and 10. Between generations 1 and 10 a positive correlation between OSP and progress of selection for PSP was found in both D-lines. The correlation coefficients r = 0.902 for the D1-line and r = 0.929 for the D2-line are both significant at the .01 level when tested against ρ = 0.

The results obtained for L-line and D-line OSPs thus strongly suggest the existence of a correlation between OSP and PSP, the most photonegative late larvae giving rise to the most dark oviposition sites preferring adults.

If there were an important environmental component accounting for the adult behaviour through pre-imaginal conditioning,this should have made DT-line OSPs different from D-line OSPs. Since DT-line newly formed

pupae were exposed to intense light (even more intense than that to which pupae of L-lines were exposed) instead of darkness, one could indeed have expected DT-females to display more light OSP through similar conditioning process. The results shown in table 37 as well as figure 11 thus militate against such an environmental conditioning. Table 37 shows that the mean OSPs of both DT-lines were rather similar to those of D-lines and figure 11 indicates that the 95 % confidence limits of DT-lines' means overlap most of the time the mean OSPs of D-lines. The shift toward lighter PSP observed in DT-lines was thus not paralleled by a shift in OSP. If one assumes that the above result relative to the comparison between D- and DT-lines OSPs pointed to a genetic component of the correlation investigated, this last result would then be in contradiction with the idea (mentioned under 2.3.12) that the DT-line genotype might have been similar to the L-line genotype.

Figure 10 shows that between generations 11 and 14 OSPs of the D-lines tended to be more photopositive than in previous generations but this was not paralleled by the trend simultaneously observed in PSP of both D-lines (see figure 4).

No mean OSP is given in table 36 for generation 0 because I omitted to record this before selection for PSP was undertaken. However, the mean OSP of twenty fe-
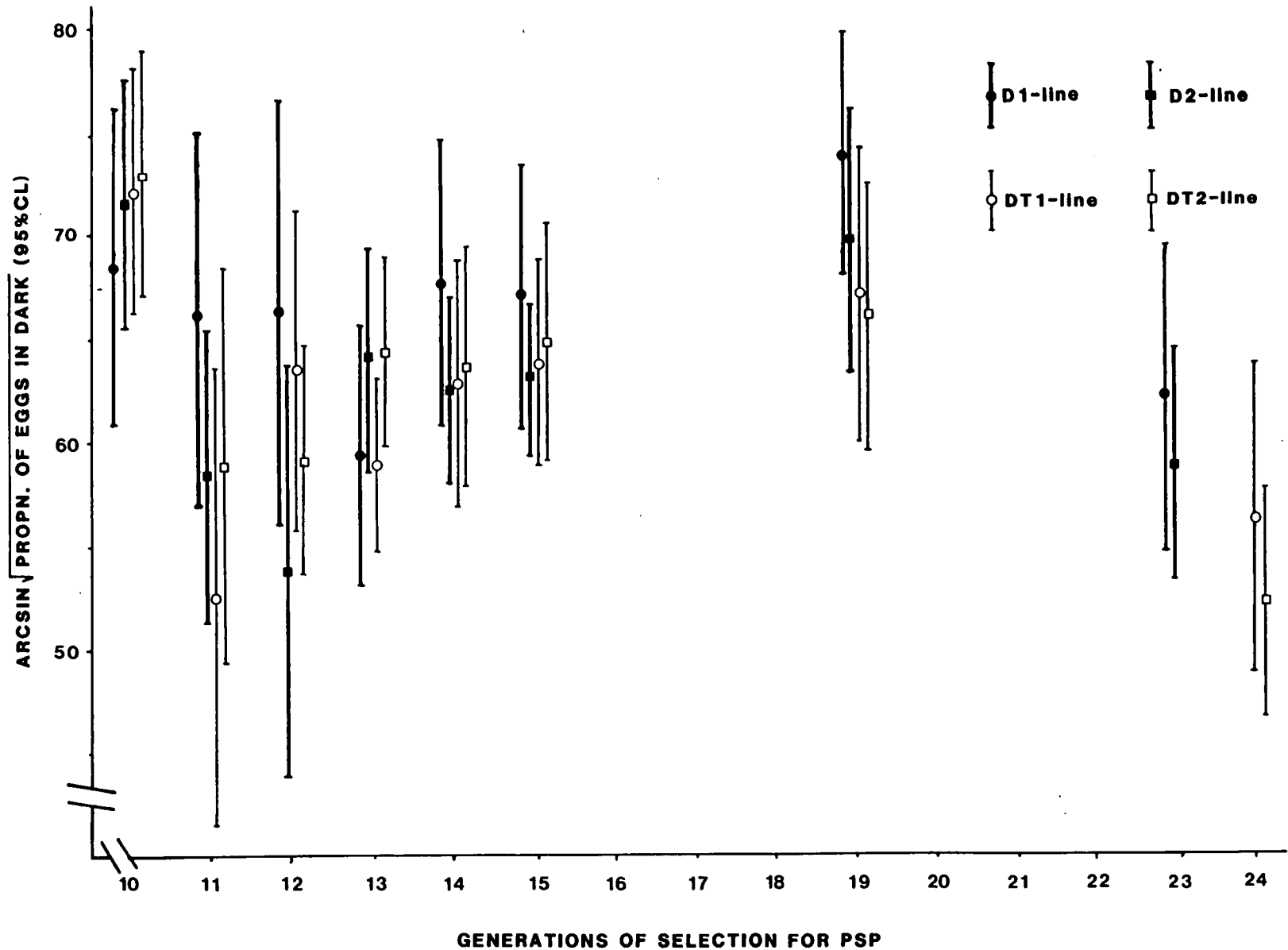
Figure 11. Oviposition site preferences (computed as in figure 10) of D- and DT-lines plotted against generations of selection for pupation site preferences.

males coming from the base population was recorded by the time the twelfth generations were being selected for PSP and a mean of 54.06 $\pm$ 3.01 was obtained, which is very close to the means reported in table 36 for the first generations of selection. This value was nevertheless not taken into account in the statistics reported in this section.

2.4 - Discussion

2.4.1 - Genetic architecture of larval phototaxis in D. simulans

According to the results of Manning and Markow (1981), natural selection can be assumed to favour positive larval photopreference at the time of pupation in D. simulans. The clear cut asymmetry observed in the response to selection for PSP in my own results thus appears to be in good accordance with this view. The control of late larval positive photopreference would then be expected to involve a good proportion of dominant genes and my results from the reciprocal hybridizations between L2- and D2-lines suggest just such genetic architecture. Besides, as Falconer (1981) points out the rapidity with which the asymmetry in response appears in the very first generations of selection may reflect a genetic asymmetry of genes having large effect on the trait

selected. Still, a maternal effect was not ruled out by the
results. Such hereditary pattern tends to suggest impli-
citely that the characters selected, or some other charac-
ters correlated with them, are components of natural fit-
ness, thus likely to show some degree of inbreeding de-
pression, with selection towards decreased fitness giving
a faster response than selection towards increased fit-
ness. This way of reasoning follows the idea that domin-
ant genes get more easily naturally selected than re-
cessive ones when they confer a selective advantage to
their carriers. Although artificial selection slightly
reduced the fecundity of the D-lines compared with that
of the L-lines,no difference in viability was found be-
tween these lines.

When introducing the hypothesis proposed in
chapter 5,I shall further discuss how the argument under-
lying the above picture of the genetic architecture in-
vestigated becomes questionable in the light of some re-
sults of the present work. At this stage I shall simply
point out some difficulties encountered by the above
picture.

If one assumes that PSP (or some other chara-
cter correlated with it) is a component of natural fitness
the apparent stability of the control-line, although poor-
ly informative, would be contradictory since it did not

indicate that the character selected was strongly subject to inbreeding depression. Furthermore, one must ask why light PSP should be a selective advantage at all. Markow (1981) argues that dark PSP may act as an adaptation against predation and dessication. As we shall see in the next chapter, D. melanogaster might then be expected to be at an advantage with respect to this character since it prefers to pupate in the dark with genes controlling such dark PSP being this time partially dominant. In relation to the question of whether genotypes can choose habitats in which they are the fittest (see under 1.2) any attempt to relate such contrast in genetic architecture of the same behaviour in two sibling species to a fitness difference might prove to be of great value.

It was mentioned earlier that variation of the standard errors on the mean responses to selection (although we saw that they are not very appropriate estimations of the sample variation) remained relatively stable throughout the whole selection experiment. Although inbreeding should have equally reduced the genetic variance in L- and D-lines, it can still be argued that in L-lines (which did not respond to selection) the loss of genetic variance brought about by artificial selection could have been less important than in D-lines. In D-lines however the observed lack of loss of phenotypic variance despite a greater expected loss of genetic variance might be explained as follows. As Falconer (1981)

emphasizes, increased homozygosity with approach to fix-
ation often results in more environmentally induced
variance than heterozygosity. This could be explained by
the different values of environmental variables at which
homozygotes have their maximal enzymic activity compared
with heterozygotes. Homozygotes with a same allelic form
of an enzyme would then maintain an adequate level of en-
zyme activity over a narrower range of environmental
variation, therefore being more sensitive to environment-
al variable than heterozygotes.

The last point relative to the response to
selection of D-lines which will be only briefly mentioned
concerns the observation that once a "plateau" was reach-
ed by generation 7 some lesser selection for dark PSP was
still possible (at least in D1-line) for a few generations.
Although this did not constitute a clear renewed response
as sometimes observed in selection experiments, this may
still suggest that some degree of recombination had
occurred between loci whose favourable alleles were origin-
ally in repulsion linkage.

2.4.2 - Correlation between preadult and adult behaviours

Like several other results in this first chap-
ter the data relative to the nature of the relationship
between larval and adult photopreferences are not con-

clusive. To predict the response to selection of a correlated character the measurement of the heritabilities of both correlated characters is required but this was done only for the response to selection for PSP and not for OSP because of lack of time. Therefore no genetic correlation can properly be measured from my data.

Nevertheless, at first sight, the existence of such a correlation is supported by the similarity in asymmetry of PSP and OSP variations between L- and D-lines as selection for PSP progressed. In effect OSP of flies selected for about twenty generations for light PSP were still undistinguishable from OSP of the base population. On the other hand OSP of D-line flies showed a significant shift towards darker preference already after four generations of selection for dark PSP. Since all the flies tested experienced the same lighting conditions for twenty-four hours before they were tested, it seems rather unlikely that the difference in OSP recorded was merely caused by differential environmental conditioning retained through this period (see also the absence of difference between OSPs of D-lines and DT-lines reported under 2.3.17).

It can therefore be suggested that the above results indicate the possibility of a genetic cause involved in the correlation observed between the two behaviours without providing conclusive evidence for it.

This led me to further investigate this possibility in
D. melanogaster as will be described in the next chapter.

2.4.3 - Effects of the traumatic environmental treatments

Table 38 summarizes the effects on PSP that
either rewarding or traumatic environmental conditions
had in the main lines studied. It must be stressed first
that apparently only trauma associated with the presence
of light altered PSP (in DT-lines), whereas trauma
associated with darkness had no effect on PSP (in the LT-
line). This may indeed suggest that the nature of the
effect has to do with some kind of photoactivation pro-
cess. Bearing in mind that the mating ability of D. si-
mulans is substantially reduced in darkness it is worth
noticing that if this caused some selective mating to
take place within LT-line, this probably did not affect
PSP.

The evidence for a genetic difference in the
control of PSP between DT-lines and D-lines was given by
the experiment reported under 2.3.5, in which subD1- and
subDT1-lines were kept under uniform light conditions for
four generations. Results of D2-line X DT1-line reciproc-
al hybridizations also supported some genetic basis to
the PSP difference between the parental flies since pro-
geny PSP were significantly different according to the

direction of the cross.

The observation that the unselected lines kept in permanent light (PL-line) did not show any change in PSP even after twenty-nine generations may seem to contradict the results found in DT-lines. Still it can be argued that it could be difficult to generate genotypes determining even more light PSP than do those genotypes present in the base population, on the ground that artificial selection in this direction had no effect at all either. Furthermore it must be stressed that PL-line was only exposed to an incident light of an irradience of 90 F.C., which was very much less than what DT-lines experienced (236 F.C.).

The crucial question to be asked then seems to be to what extent DT-line average genotype was maybe becoming more similar to L-line average genotype as the trauma was further applied. As already mentioned,I shall advocate the view that the induced similarity in PSP between DT- and L-lines was not just due to some increased resemblance between average frequency of alleles influencing light PSP on the following grounds:

a) The comparison of mortality rates and fecundity rates did not indicate that intra-line selection was operating differentially in DT- and D-lines. Indeed if such mechanism was responsible for the above dis-

crepancy it would have to have been very marked to bring about a significant divergence in two or three generations. For instance the absence of an overall increased fecundity of DT-line flies comparatively to D-line flies fecundity would be difficult to explain on such basis.

b) Mean OSPs of DT-lines were not different from mean OSPs of D-lines. Assuming that a genetic correlation existed between PSP and OSP, the photopreferences at the time of oviposition of DT-lines should have been more like those of L-lines. Besides, the slight trend towards more dark PSP shown by both DT-lines at the end of the experiment would also be hard to understand since OSPs of the same lines simultaneously showed the opposite trend.

c) Lastly results from cross experiments between L2-line and D2-line have shown that dominant X-linked genes with large effect are likely to determine light PSP. With respect to this it can be argued that if the change in PSP of DT-lines (comparatively to D-lines) was primarily determined by the gradual increase of the frequency of the above X-linked allele(s), DT-line females should then have been more likely to get at least one such light PSP determining allele than males since they carry two X-chromosomes. As a phenotypic consequence more female than male larvae should have sought light

pupation sites thus causing a sex ratio rather biased towards males among the selected pupae in DT-lines (selected for dark PSP). Table 34 indicates that this was not the case and there was even a weak trend (but consistent between replicates) towards a sex ratio biased towards females.

2.4.4 - Conclusion

It would be very premature to speculate at this stage on possible alternative explanations accounting for the contradictions listed in this discussion. A full consideration will not be attempted until chapter 5, after more relevant results have been presented. From this first set of experiments using D. simulans it appeared that the design used for the measurement of larval phototaxis could be improved at least in the three following aspects.

a) The weak attractive effect that the lateral plugs of the gradient apparatus exerted on larvae must be suppressed.

b) The accuracy of the counting procedure for eggs to be deposited in the gradients must be improved.

c) The possibility that different populations of yeast and bacteria between lines were repeated-

ly passed on through the strips of medium containing the eggs must be suppressed. At the end of the selection experiment it was observed that the texture of the surface of the medium in the boxes was no longer identical between lines at the time PSP was assessed. This might have been due to the presence of slightly different "microbial luggage" carried by lines exposed to different environmental conditions, although it will be shown in the next chapter that it is very unlikely that this substantially interfered with larval photopreference.

Table 1

Cumulated mean responses to selection of L1-lines and L2-lines calculated over the first ten generations of selection and adjusted selection differentials.

generations of selection	cumulated mean responses		adjusted selection differentials	
	L1-line	L2-line	L1-line	L2-line
1	3.7	4.3	42.5	48.0
2	7.0	8.7	47.1	46.5
3	4.1	- 1.9	42.5	39.7
4	1.1	- 0.4	46.7	52.5
5	5.8	4.4	48.4	51.0
6	11.5	4.0	43.7	46.4
7	5.4	- 7.5	33.9	39.0
8	10.0	6.8	56.2	58.3
9	- 4.3	1.3	35.4	39.0
10	7.5	11.5	54.2	49.5

Table 2

Intra - L1-line and intra-L2-line PSP variations.

The median test was used to compare pairs of box distributions and probability values p were determined by a χ^2 table.

generations of selection	p (intra-L1-line)	p (intra-L2-line)
1	< .05 *	< .9
2	< .05 *	< .001 *
3	< .9	< .5
4	> .9	< .9
5	< .9	< .9
6	< .9	< .05 *
7	< .5	< .3
8	< .01 *	< .1
9	< .5	< .001 *
10	< .3	< .3
11	< .3	< .2
12	< .001 *	< .1
13	< .1	< .9
14	< .9	< .2
15	< .9	< .1
17	> .9	< .3
18	< .5	< .2
19	< .2	< .01 *
20	< .01 *	< .5
21	< .5	< .9
22	< .9	< .9
23	< .9	< .9
24	< .5	

* indicates a difference significant at p<.05.

Table 3

PSP variation between L1-line and L2-line.

Median test and probability values p as in table 2.

generations of selection	P (L1'vs L2')	P (L1'vs L2'')	P (L1''vs L2')	P (L1''vs L2'')
1	< .5	< .2	< .3	< .5
2	< .2	< .01 *	< .01 *	< .5
3	< .05 *	< .01 *	< .5	< .01 *
4	> .9	< .9	> .9	< .9
5	< .5	< .9	< .5	< .9
6	< .5	< .05 *	< .9	< .001 *
7	< .5	~ .9	< .2	< .5
8	< .1	> .9	< .05 *	< .3
9	< .5	~ .05 *	< .9	< .01 *
10	< .05 *	~ .01 *	< .3	< .3
11	< .3	< .9	> .9	< .5
12	< .2	< .9	< .001 *	< .05 *

* indicates a difference significant at $p \leqslant .05$.

Table 4

PSP variation between L1-line and LT-line.
Median test and probability values p as in table 2.
        (selection was relaxed at generation 25)

generations of selection	p(L1'vs LT')	p(L1'vs LT'')	p(L1''vs LT')	p(L1''vs LT'')
23	< .05	~ .01	< .9	< .05
24	< .5	< .2	< .5	< .05
26	< .5	> .9	< .5	< .9
27	< .5	~ .9	< .9	< .9
28	< .9	< .3	< .9	< .9

Table 5

Cumulated mean responses to selection of D1- and D2-lines calculated over the first seven generations of selection and selection differentials.

generations of selection	cumulated mean responses		selection differentials	
	D1-line	D2-line	D1-line	D2-line
1	6.8	11.1	39.2	39.2
2	6.6	10.4	32.4	28.1
3	18.0	14.2	32.6	28.8
4	21.5	23.8	21.2	25.0
5	21.8	24.9	17.7	15.4
6	29.4	32.3	17.4	14.3
7	25.9	27.5	9.8	6.9

Table 6

Intra-D1-line and intra-D2-line PSP variations.
Median test and probability values p as in table 2.

generations of selection	p(intra-D1-line)	p(intra-D2-line)
1	> .9	< .5
2	< .9	< .2
3	< .5	< .5
4	< .5	< .5
5	< .9	< .9
6	< .9	> .9
7	< .5	< .3
8	< .3	< .2
9	< .9	< .2
10	< .9	< .05 *
11	< .2	< .9
12	> .9	< .1
13	~ .01 *	< .05 *
14	< .5	< .9
15	< .9	< .5
17	< .3	< .05 *
18	< .5	< .3
19	~ .001 *	< .9
20	< .3	< .9
21	< .5	< .9
22	< .001 *	< .9
23	< .9	< .3
24	-	< .5

* indicates a difference significant at p < .05.

Table 7

PSP variation between D1-line and D2-line.
Median test and probability values p as in table 2.

generations of selection	P (D1'vs D2')	P (D1'vs D2'')	P (D1''vs D2')	P (D1''vs D2'')
1	< .3	< .05 *	< .3	< .1
2	< .9	< .5	< .01 *	∿ .5
3	< .9	< .2	< .2	< .02 *
4	< .5	> .9	< .9	< .5
5	< .1	< .2	< .3	< .5
6	< .1	< .1	< .3	< .2
7	< .5	< .3	> .9	< .9
8	< .1	< .9	< .9	< .5
9	< .1	< .9	< .2	> .9
10	< .2	< .9	< .1	< .9
11	< .05 *	< .05 *	< .9	< .9
12	< .2	< .3	< .2	< .3

* indicates a difference significant at p < .05.

Table 8

PSP variation between L1-line and D1-line.
Median test and probability values p as in table 2.

generations of selection	p(L1'vsD1')	p(L1'vsD1'')	p(L1''vs D1')	p(L1''vs D1'')
1	< .9	< .5	< .01	< .02
2	< .001	< .001	< .001	< .001
3	< .001	< .001	< .001	< .001
4	< .001	< .001	< .001	< .001
5	< .001	< .001	< .001	< .001
6.	< .001	< .001	< .001	< .001

Table 9

PSP variation between L1-line and D2-line.
Median test and probability p as in table 2.

generations of selection	p(L1'vs D2')	p(L1'vs D2'')	p(L1'' vs D2')	p(L1''vs D2'')
1	< .3	< .01	< .01	< .001
2	< .01	< .001	< .05	< .001
3	< .001	< .001	< .001	< .001
4	< .001	< .001	< .001	< .001
5	< .001	< .001	< .001	< .001
6	< .001	< .001	< .001	< .001

Table 10

PSP variation between L2-line and D1-line.
Median test and probability values p as in table 2.

generations of selection	p(L2'vs D1')	p(L2'vs D1'')	p(L2''vs D1')	p(L2''vs D1'')
1	< .05	< .05	< .01	< .01
2	< .001	< .001	< .001	< .001
3	< .001	< .001	< .01	< .001
4	< .001	< .001	< .001	< .001
5	< .001	< .001	< .001	< .001
6	< .001	< .001	< .001	< .001

Table 11

PSP variation between L2-line and D2-line.
Median test and probability values p as in table 2.

generations of selection	p(L2'vs D2')	p(L2'vs D2'')	p(L2''vs D2')	p(L2''vs D2'')
1	< .01	< .001	< .01	< .001
2	< .001	< .001	< .01	< .001
3	< .001	< .01	< .05	< .2
4	< .001	< .001	< .001	< .001
5	< .001	< .001	< .001	< .001
6	< .001	< .001	< .001	< .001

Table 12

Intra - DT1-line and intra-DT2-line PSP variations.
Median test and probability values p as in table 2.

generations of selection	p(intra-DT1-line)	p(intra-DT2-line)
6	< .9	-
7	< .05 *	-
8	< .5	-
9	< .2	< .9
10	< .3	< .05 *
11	< .9	< .9
12	< .1	< .1
13	< .001 *	< .001 *
14	< .1	∼.1
15	< .5	< .2
17	< .01 *	< .2
18	< .5	< .3
19	< .05 *	< .001 *
20	< .9	< .9
21	> .9	< .5
22	< .9	∼.9
23	∼.05 *	< .2
24	< .3	-

* indicates a difference significant at p < .05.

Table 13

PSP variation between DT1-line and DT2-line.
Median test and probability values p as in table 2.

generations of selection	p (DT1'vs DT2')	p (DT1'vs DT2'')	p (DT1''vs DT2')	p (DT1''vs DT2'')
14	< .05 *	< .05 *	< .9	< .5
15	< .9	< .2	< .5	< .5
17	< .9	< .9	< .2	< .001 *
18	< .2	< .2	< .5	< .9
19	< .001 *	< .5	< .001 *	< .5
20	< .5	< .2	< .3	< .05 *
21	< .9	< .9	> .9	< .5

* indicates a difference significant at p < .05.

Table 14

PSP variation between D1-line and DT1-line.
Median test and probability values p as in table 2.

generations of selection	P (D1'vs DT1')	P (D1'vs DT1'')	P (D1''vsDT1')	P (D1''vs DT1'')
6	< .9	< .9	< .2	~ .9
7	< .01 *	< .001 *	< .001 *	< .001 *
8	< .2	< .5	< .05 *	< .5
9	< .2	< .05 *	< .5	< .1
10	< .001 *	< .01 *	< .001 *	< .001 *
11	< .001 *	< .01 *	< .05 *	< .5
12	< .01 *	< .2	< .01 *	< .2
13	< .001 *	< .01 *	< .01 *	< .7
14	< .001 *	< .001 *	< .001 *	< .001 *
15	< .001 *	< .001 *	< .001 *	< .001 *
17	< .01 *	~ .05 *	< .001 *	< .001 *
18	< .001 *	< .001 *	< .001 *	< .001 *
19	< .001 *	< .001 *	< .001 *	< .001 *
20	< .001 *	< .001 *	< .001 *	< .001 *
21	< .001 *	< .001 *	< .001 *	< .001 *
22	< .01 *	< .05 *	< .001 *	< .001 *
23	< .001 *	< .01 *	< .001 *	< .01 *

* indicates a difference significant at $p \leqslant .05$.

Table 15

PSP variation between D1-line and DT2-line.
Median test and probability values p as in table 2.

generations of selection	P (D1'vs DT2')	P (D1'vs DT2'')	P (D1''vs DT2')	P (D1''vs DT2'')
9	< .9	~.9	< .5	< .9
10	< .01 *	< .001 *	< .001 *	< .001 *
11	< .001 *	< .001 *	< .1	< .1
12	< .3	< .02 *	< .3	< .02 *
13	< .001 *	< .001 *	< .001 *	< .02 *
14	< .001 *	< .001 *	< .001 *	< .001 *
15	< .001 *	< .001 *	< .001 *	< .001 *
17	< .01 *	< .001 *	< .001 *	< .001 *
18	< .1	< .001 *	< .02 *	< .001 *
19	< .05 *	< .001 *	< .001 *	< .001 *
20	< .001 *	< .001 *	< .001 *	< .001 *
21	< .01 *	< .001 *	< .001 *	< .001 *
22	< .2	< .5	< .001 *	< .001 *
23	< .001 *	< .001 *	< .001 *	< .001 *

* indicates a difference significant at p < .05.

Table 16

PSP variation between D2-line and DT1-line.
Median test and probability values p as in table 2.

generations of selection	p (D2'vs DT1')	p (D2'vs DT1'')	p (D2''vs DT1')	p (D2''vs DT1'')
6	< .1	< .2	< .1	< .2
7	< .001 *	< .001 *	< .001 *	< .001 *
8	< .05 *	< .2	< .02 *	< .2
9	< .9	< .2	< .3	< .05 *
10	< .01 *	< .01 *	< .001 *	< .001 *
11	< .1	< .5	< .2	< .9
12	< .001 *	< .05 *	< .1	< .9
13	< .001 *	< .5	< .01 *	< .9
14	< .001 *	< .001 *	< .01 *	< .001 *
15	< .001 *	< .001 *	< .001 *	< .001 *
17	< .001 *	~ .3	< .001 *	< .001 *
18	< .001 *	< .001 *	< .001 *	< .001 *
19	< .001 *	< .001 *	< .001 *	< .001 *
20	< .001 *	< .001 *	< .001 *	< .001 *
21	< .001 *	< .001 *	< .001 *	< .001 *
22	< .001 *	< .001 *	< .001 *	< .001 *
23	< .001 *	< .05 *	< .001 *	< .3
24	< .001 *	< .001 *	< .001 *	< .001 *

* indicates a difference significant at p < .05.

Table 17

PSP variation between D2-line and DT2-line.
Median test and probability values p as in table 2.

generations of selection	P (D2'vs DT2')	P (D2'vs DT2'')	P (D2''vs DT2')	P (D2''vs DT2'')
9	< .05 *	< .2	< .5	< .9
10	< .01 *	< .001 *	< .001 *	< .001 *
11	< .2	< .2	< .3	< .3
12	< .1	< .001 *	> .9	< .2
13	< .001 *	< .05 *	< .001 *	< .01 *
14	< .001 *	< .001 *	< .001 *	< .001 *
15	< .001 *	< .001 *	< .001 *	< .001 *
17	< .05 *	< .001 *	< .001 *	< .001 *
18	< .001 *	< .001 *	< .02 *	< .001 *
19	< .001 *	< .001 *	< .001 *	< .001 *
20	< .001 *	< .001 *	< .001 *	< .001 *
21	∿ .02 *	< .001 *	< .02 *	< .001 *
22	< .001 *	< .01 *	< .001 *	< .01 *
23	< .001 *	< .001 *	< .001 *	< .001 *

* indicates a difference significant at p < .05.

Table 18

Cumulated mean "responses" to selection with trauma (measured as trends towards light PSP) of DT1-line and DT2-line.
Corresponding selection differentials and slopes of successive responses.

generations of selection	cumulated mean responses		selection differentials		slopes of successive responses	
	DT1-line	DT2-line	DT1-line	DT2-line	DT1-line	DT2-line
7	14.8	-	11.5	-	14.8	-
8	21.1	-	26.3	-	6.3	-
9	16.1	-	32.6	-	- 5.0	-
10	20.1	14.8	27.6	20.6	4.0	14.8
11	16.0	7.7	31.6	35.4	- 4.1	- 7.1
12	18.9	7.6	27.5	28.3	2.9	- 0.1
13	15.5	19.6	30.4	28.2	- 3.4	12. 0
14	25.8	18.5	27.0	40.2	10.3	- 1.1
15	36.4	23.0	37.3	39.1	10.6	4.5

Table 19

PSP variation between sub D1-line and sub DT1-line kept both under identical conditions of light (12 hour dark/12 hour reduced light cycle) with no selection applied.
Median test and probability values p as in table 2.

generation number	p (D1'vs DT1')	p (D1'vs DT1'')	p (D1''vs DT1')	p (D1''vs DT1'')
19	< .001	< .001	< .001	< .001
20	< .001	< .001	< .001	< .001
21	< .001	< .001	< .001	< .001
22	< .001	< .001	< .001	< .001

Table 20

PSP variation between DT1-line and DTD-line.
Median test and probability values p as in table 2.

(selection was relaxed at generation 26)

generations of selection	p (DT1'vs DTD')	p (DT1'vs DTD'')	p (DT1''vs DTD')	p (DT1''vs DTD'')
23	< .01 *	< .001 *	< .5	< .1
24	< .01 *	< .001 *	< .3	< .01 *
25	~ .01 *	< .01 *	< .05 *	< .01 *
27	< .001 *	< .001 *	< .001 *	< .001 *
28	< .001 *	< .001 *	< .5	< .5
29	< .1	< .02 *	< .1	< .01 *

* indicates a difference significant at p < .05.

Table 21

PSP variation between PL- and PD- unselected lines respectively kept under permanent light and permanent darkness.
Median test and probability values p as in table 2.

generation number	(PL'vs PD')	(PL'vs PD'')	(PL''vs PD')	(PL''vs PD'')
14	< .01*	> .9	< .2	< .9
19	< .2	< .3	-	-
29	< .9	< .3	< .01*	< .5

* indicates a difference significant at p < .01.

Table 22

PSP of the control-line (C-line) and variation between generation 3 and 5. Median test and probability values p as in table 2 (4 boxes were run at generation 3 and 5).

generation	mean PSP (%)
Parental O	60.8
Unselected control gener. 3 (C3)	59.3
Unselected control gener. 5 (C5)	64.0

Variation C_3 vs C_5	p
C_3' vs C_5'	< .5
C_3' vs C_5''	< .3
C_3' vs C_5'''	< .2
C_3' vs C_5''''	< .5
C_3'' vs C_5'	< .1
C_3'' vs C_5''	< .9
C_3'' vs C_5'''	< .05 *
C_3'' vs C_5''''	< .2
C_3''' vs C_5'	< .3
C_3''' vs C_5''	< .9
C_3''' vs C_5'''	< .1
C_3''' vs C_5''''	< .5
C_3'''' vs C_5'	< .3
C_3'''' vs C_5''	< .9
C_3'''' vs C_5'''	< .01 *
C_3'''' vs C_5''''	< .2

* indicates a difference significant at $p < .05$.

Table 23

Comparison of pupation site distribution of larvae run in a "control gradient apparatus" with a homogeneous distribution (2 x 10 contingency tables were used).

	x^2	df	P
box 1	12.250	9	~ .2
box 2	13.725	9	< .2

Table 24

Reciprocal hybridizations between L2-line and D2-line.
Mean PSP of progeny (% in dark).

♂

	L2	D2
L2	54.0	55.4
D2	76.6	84.9

♀

generation 22

♂

	L2	D2
L2	55.1	56.4
D2	65.3	83.3

♀
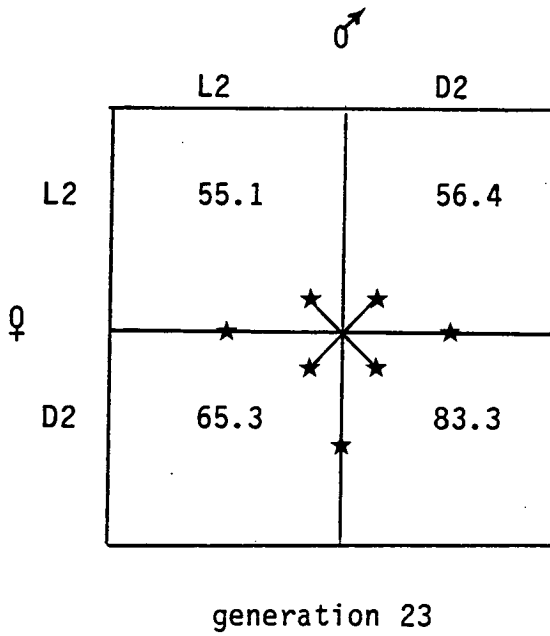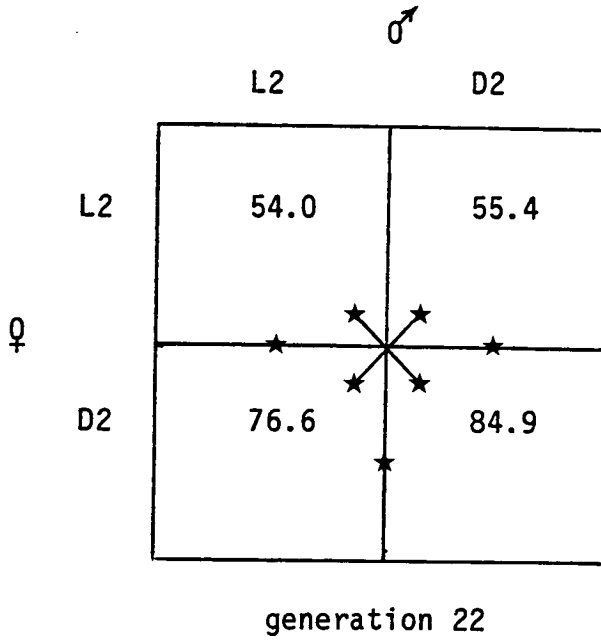
generation 23

★ indicates a difference significant at the .05 level, using the
median test.

Table 25

PSP variation between progeny issued from the reciprocal hybridizations between L2-line x D2-line at generation 22 of selection. Median test and probability values p as in table 2.

variation between	P (LD' vs DL')	P (LD' vs DL'')	P (LD'' vs DL')	P (LD'' vs DL'')
♀ L2 x ♂ D2 progeny				
and	< .001	< .001	< .001	< .001
♀ D2 x ♂ L2 progeny				

variation between	P (LD' vs L')	P (LD' vs L'')	P (LD'' vs L')	P (LD'' vs L'')
♀ L2 x ♂ D2 progeny				
and	> .9	< .9	< .2	< .5
L2				

variation between	P (LD' vs D')	P (LD' vs D'')	P (LD'' vs D')	P (LD'' vs D'')
♀ L2 x ♂ D2 progeny				
and	< .001	< .001	< .001	< .001
D2				

variation between	P (DL' vs L')	P (DL' vs L'')	P (DL'' vs L')	P (DL'' vs L'')
♀ D2 x ♂ L2 progeny				
and	< .001	< .001	< .001	< .001
L2				

variation between	P (DL' vs D')	P (DL' vs D'')	P (DL'' vs D')	P (DL'' vs D'')
♀ D2 x ♂ L2 progeny				
and	< .001	< .001	< .001	< .01
D2				

Table 26

PSP variation between progeny issued from the reciprocal hybridizations between L2-line and D2-line at generation 23 of selection. Median test and probability values p as in table 2.

variation between ♀ L2 x ♂ D2 progeny	P (LD'vs DL')	P (LD'vs DL'')	P (LD''vs DL')	P (LD''vs DL'')
and ♀ D2 x ♂ L2 progeny	< .01	< .02	< .02	~ .2

variation between ♀ L2 x ♂ D2 progeny	P (LD'vs L')	P (LD'vs L'')	P (LD''vs L')	P (LD''vs L'')
and L2	< .9	< .5	~ .3	< .5

variation between ♀ L2 x ♂ D2 progeny	P (LD'vs D')	P (LD'vs D'')	P (LD''vs D')	P (LD''vs D'')
and D2	< .001	< .001	< .001	< .001

variation between ♀ D2 x ♂ L2 progeny	P (DL'vs L')	P (DL'vs L'')	P (DL''vs L')	P (DL''vs L'')
and L2	< .001	< .001	< .001	< .001

variation between ♀ D2 x ♂ L2 progeny	P (DL'vs D')	P (DL'vs D'')	P (DL'' vs D')	P (DL'' vs D'')
and D2	< .001	< .001	< .001	< .001

Table 27

Reciprocal hybr .izations between D2-line and DT1-line.
Mean PSP of prc .ny (% in dark).

★ indicates a d ference significant at the .05 level, using the
median test.

TABLE 28

PSP variation between progeny issued from the reciprocal hybridizations between D2-line and DT1-line at generation 24 of selection. Median test and probability values p as in table 2.

variation between ♀ D2 x ♂ DT1 progeny	p(D,DT'vs DT,D')	p(D,DT'vs DT,D'')	p(D,DT''vs DT,D')	p(D,DT''vs DT,D'')
and ♀ DT1 x ♂ D2 progeny	< .3	< .01	< .02	< .001

variation between ♀ D2 x ♂ DT1 progeny	p(D,DT'vs D')	p(D,DT'vs D'')	p(D,DT''vs D')	p(D,DT''vs D'')
and D2	< .1	< .3	< .3	< .3

variation between ♀ D2 x ♂ DT1 progeny	p(D,DT'vs DT')	p(D,DT'vs DT'')	p(D,DT''vs DT')	p(D,DT''vs DT'')
and DT1	< .05	< .5	< .01	< .2

variation between ♀ DT1 x ♂ D2 progeny	p(DT,D'vs D')	p(DT,D'vs D'')	p(DT,D''vs D')	p(DT'',D vs D'')
and D2	< .001	< .01	< .001	< .001

variation between ♀ DT1 x ♂ D2 progeny	p(DT,D' vs DT')	p(DT,D' vs DT'')	p(DT,D'' vs DT')	p(DT,D''vs DT'')
and DT1	< .2	< .9	< .3	~ .001

Table 29

Comparison of fecundities of L-lines and D-lines. T-test on mean number of eggs laid per female per 24 hours. Probability values p were determined by a t-table. NS indicates that p is > .05.

generations of selection	P(L1 vs D1)	P(L1 vs D2)	P(L2 vs D1)	P(L2 vs D2)
4	< .05	< .025	~ .05	< .025
5	< .01	< .005	< .001	< .001
6	< .005	< .001	NS	NS
7	NS	NS	NS	NS
8	NS	NS(< .1)	NS	NS
9	NS	< .05	< .05	< .001
10	< .001	< .001	< .001	< .001

Table 30

Comparison of fecundities of D-lines and DT-lines. T-test on mean number of eggs laid per females per 24 hours. Probability values p were determined by a t-table.

generations of selection	P(D1 vs DT1)	P(D1 vs DT2)	P(D2 vs DT1)	P(D2 vs DT2)
10	< .9	< .001 *	< .3	< .2
11	< .1	< .01 *	< .4	~ .1
12	< .4	< .2	< .9	< .4
13	< .05 *	< .3	< .05 *	< .3
14	< .1	< .3	< .2	< .4

* indicates a difference significant at p < .05.

Table 31

Mean number of eggs ($\bar{x}$) laid per female per 24 hours and standard deviation (s) in D-lines and DT-lines at the time the two lines separated.

generations of selection	D1-line		D2-line		DT1-line		DT2-line	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
10	13.9	7.0	20.3	9.7	15.9	12.4	24.5	8.3
11	16.6	6.8	18.8	10.2	21.5	7.4	24.5	8.8
12	21.2	6.7	22.3	7.1	23.2	6.2	24.4	4.7
13	22.0	6.9	22.3	7.3	27.3	7.0	24.8	7.4
14	22.4	6.5	23.2	6.8	26.4	6.2	25.2	7.2
totals s:	33.9		41.1		39.2		36.4	

Table 32

Mean egg - larval mortality ($\bar{x}$) of L-, D- and DT-lines, and number of eggs examined (n) calculated up to generation 23 of the selection experiment.

selected lines	$\bar{x}$ (%)	n
L1-line	4.7	6600
L2-line	8.4	6600
D1-line	7.5	6600
D2-line	6.8	6600
DT1-line	6.4	5100
DT2-line	10.2	4200

Table 33

Mean mortality at the pupal stage ($\bar{x}$) of L-, D- and DT-lines and number of pupae examined (n).

selected line	$\bar{x}$ (%)	n
L1-line	2.8	600
L2-line	5.2	600
D1-line	3.2	840
D2-line	4.8	880
DT1-line	4.2	480
DT2-line	4.8	440

Table 34

Percentages of males found among the flies emerging from the selected pupae of L-, D- and DT-lines. Probability values p that these percentages differ from 50 % were determined by a standard normal table.

selected line	number of flies examined	percent males	p
L1-line	583	51.6	~.44
L2-line	569	53.2	~.13
D1-line	813	51.8	~.30
D2-line	838	51.4	~.42
DT1-line	460	46.7	~.16
DT2-line	419	47.3	~.27

Table 35

Comparison between the sex ratio of the flies emerging from the selected pupae in D-lines and the sex ratio of the flies emerging from the selected pupae in DT-lines. Probability values p were determined by a χ^2 table, from 2 x 2 contingency tables.

selected lines compared	P
D1 vs DT1	< .1
D1 vs DT2	< .2
D2 vs DT1	< .2
D2 vs DT2	< .2

Table 36

Oviposition site preferences (OSPs) of L- and D-lines.
Mean values of arcsin √proportion of eggs laid in dark by females
selected for light and dark PSPs and 95 % confidence limits (C.L.).

generations of selection	L1-line OSP	95 % C.L.	L2-line OSP	95 % C.L.	D1-line OSP	95 % C.L.	D2-line OSP	95 % C.L.
1	52.45	± 4.71	-	-	52.71	± 4.58	-	-
2	51.82	± 4.13	50.72	± 3.88	53.20	± 4.75	52.46	± 4.35
3	51.62	± 4.58	54.80	± 5.69	58.48	± 5.57	59.36	± 6.05
4	49.06	± 5.40	49.21	± 5.22	63.84	± 6.30	60.45	± 5.33
5	46.90	± 4.31	52.55	± 6.95	65.43	± 6.78	65.84	± 6.31
6	49.89	± 9.26	46.42	± 3.59	62.19	± 5.98	64.48	± 8.56
7	49.89	± 4.22	52.31	± 3.40	64.08	± 5.66	64.10	± 5.59
8	49.49	± 4.48	48.36	± 4.50	65.95	± 4.03	66.05	± 5.57
9	43.63	± 3.52	52.77	± 4.58	66.63	± 5.24	70.10	± 6.23
10	51.04	± 4.22	52.91	± 4.43	68.79	± 7.82	71.59	± 5.91
11	44.15	± 5.13	50.89	± 5.31	66.09	± 9.26	58.30	± 7.10
12	46.47	± 6.33	51.73	± 9.72	66.34	+10.34	53.77	± 9.97
13	46.40	± 4.49	44.77	± 4.20	59.80	± 6.45	64.02	± 5.44
14	48.00	± 4.46	55.91	± 6.44	67.74	± 7.05	62.50	± 4.64
15	45.83	± 4.60	45.12	± 4.30	67.10	± 6.50	63.06	± 3.72
19	53.39	± 6.37	54.49	± 6.38	73.87	± 5.87	69.71	± 6.38
23	56.28	± 7.11	54.91	± 3.24	62.03	± 7.35	58.66	± 5.59

Table 37

Oviposition site preferences (OSPs) of D- and DT-lines.
Mean values of arcsin √proportion of eggs laid in dark by females
selected for dark PSP experiencing either traumatic or rewarding
environmental conditions and 95 % confidence limits (C.L.).

generations of selection	D1-line OSP	95 % C.L.	D2-line OSP	95 % C.L.	DT1-line OSP	95 % C.L.	DT2-line OSP	95 % C.L.
10	68.79	± 7.82	71.59	± 5.91	72.06	± 6.04	72.95	± 6.01
11	66.09	± 9.26	58.30	± 7.10	52.44	± 11.12	58.81	± 9.52
12	66.34	± 10.34	53.77	± 9.97	63.50	± 7.87	59.04	± 5.64
13	59.18	± 6.45	64.02	± 5.44	58.93	± 4.24	64.33	± 4.55
14	67.74	± 7.05	62.50	± 4.64	62.77	± 5.96	63.59	± 5.88
15	67.10	± 6.50	63.06	± 3.72	63.72	± 5.08	64.79	± 5.84
19	73.87	± 5.87	69.71	± 6.38	67.03	± 7.10	66.02	± 6.52
23	62.03	± 7.35	58.66	± 5.59	-	-	-	-
24	-	-	-	-	56.13	± 7.53	52.10	± 5.64

Table 38

Summary of the changes in PSP through generations in the main lines
studied. Estimates of the "changes" are measured as responses (R)
to selection over the generations listed.

lines	change in PSP (R)	generations over which R was calculated
L1-line	no change	1 - 10
L2-line	no change	1 - 10
LT-line	no change	1 - 5
PL-line	no change	1 - 29
D1-line	4.088	1 - 7
D2-line	4.186	1 - 7
DT1-line	- 2.309	1 - 10 (6-15)
DT2-line	- 2.619	1 - 10 (6-15)
PD-line	no change	1 - 29

CHAPTER 3 : PHOTOPREFERENCES IN D. MELANOGASTER

3.1 - Introduction

3.1.1 - Justification of the choice of the species used

It was emphasized in the previous chapter that in contrast with D. simulans, D. melanogaster is light independent as far as its mating ability goes. Since this difference, which is likely to reflect a difference in the genetic architecture underlying this ability might also affect other aspects of phototactic behaviour (as alluded to in 2.1.1), I decided to collect data in similar experimental conditions with this second species. The already mentioned higher photonegativity observed in experiments with D. melanogaster relative to D. simulans has again been mainly investigated in adults, although observations on larval phototaxis have been reported more recently (Manning and Markow, 1981). Besides, the availability of various balancer stocks carrying marker genes makes it easier to dissect phototactic behaviour genetically in D. melanogaster and the results of chapter 2 showed the need for analyzing phototaxis at the genetic level.

3.1.2 - <u>Previous results on phototaxis in D. melanogaster</u>

It was also mentioned earlier that variance in phototaxis appears to be strongly sex-linked in <u>D</u>. <u>melanogaster</u> adults, in contrast with <u>D</u>. <u>simulans</u>. By assuming that in short-term selection experiments recombination and segregation are the two chief sources of new genetic variation, Markow (1975**a**) observed that in most strains selected in the presence of multiple inversions (used to suppress genetic recombination) the X chromosome was important regardless of conditions which restricted genetic recombination during selection.

In many selection experiments for adult photo-preference a greater response to selection for light than for dark preference was often recorded. Although there may be several causes for such asymmetry in divergence (see Falconer, 1981), such results are in accordance with Walton's (1970) finding that sex-linked genes determining negative phototaxis are at least partly dominant, having been favoured by natural selection. Furthermore, it appears that not only the X chromosome influences adult phototaxis, and indeed Markow (1975**b**) reached the conclusion that polygenes influencing phototactic behaviour in <u>D</u>. <u>melanogaster</u> are probably located on all chromosomes.

Larval phototaxis may also be chiefly controlled by X-linked genes ; Manning and Markow (1981) invest-

igated PSPs of progeny issued from reciprocal hybridiz-
ations between D. melanogaster and D. simulans. They
found that progeny from the cross of D. melanogaster
females with D. simulans males showed PSP intermediate
between those of the two parental species, while the re-
ciprocal cross offspring showed more light PSP, which
agrees with the postulate of an involvement of a sex-link-
ed locus. Interestingly, the latter authors point out in
the same study that during the first two larval instars
D. melanogaster is markedly more photopositive than D.
simulans. The results presented in chapter 2 relative to
this latter species also suggested sex linkage unless the
trait was under the influence of maternal effect.

3.2 - Methods

3.2.1 - Selection procedure

In this second set of experiments using D. mela-
nogaster the pattern of selection conformed to the general
pattern shown in Figure 1 (p. 38). This means that "re-
warded" and "traumatic" lines were selected right from the
beginning of the experiment and were always run simultane-
ously in the light gradient apparatus.

3.2.2 - Strain and medium used

Thirty $\underline{D}$. $\underline{melanogaster}$ females of a Death Valley population recently initiated from a large collection made by Dr. L. Nunney in California were pooled together with thirty females of a wild stock (about two hundred and fifty females caught near Leeds by Dr. S. Newbury, five months before the beginning of the experiment). The flies were kept for three generations in a population cage at 25°C to form the base population. Standard medium as described under 2.2.2 was used throughout, and all experiments were performed at 25°C $\pm$ 1°C. CO_2 was used as anaesthetic until generation 22 of selection and was thereafter replaced by ether because CO_2 cylinders were no longer available in my laboratory.

3.2.3 - Light gradient apparatus used for larval photopreference

A different design was devised to assess PSP, for reasons mentioned at the end of the last chapter. I also intended to handle more lines at the same time and therefore a less time consuming design was needed (requiring less preparation of medium and less washing up). At each generation PSPs were then recorded for each line in three square light gradients, much smaller than the previous ones.
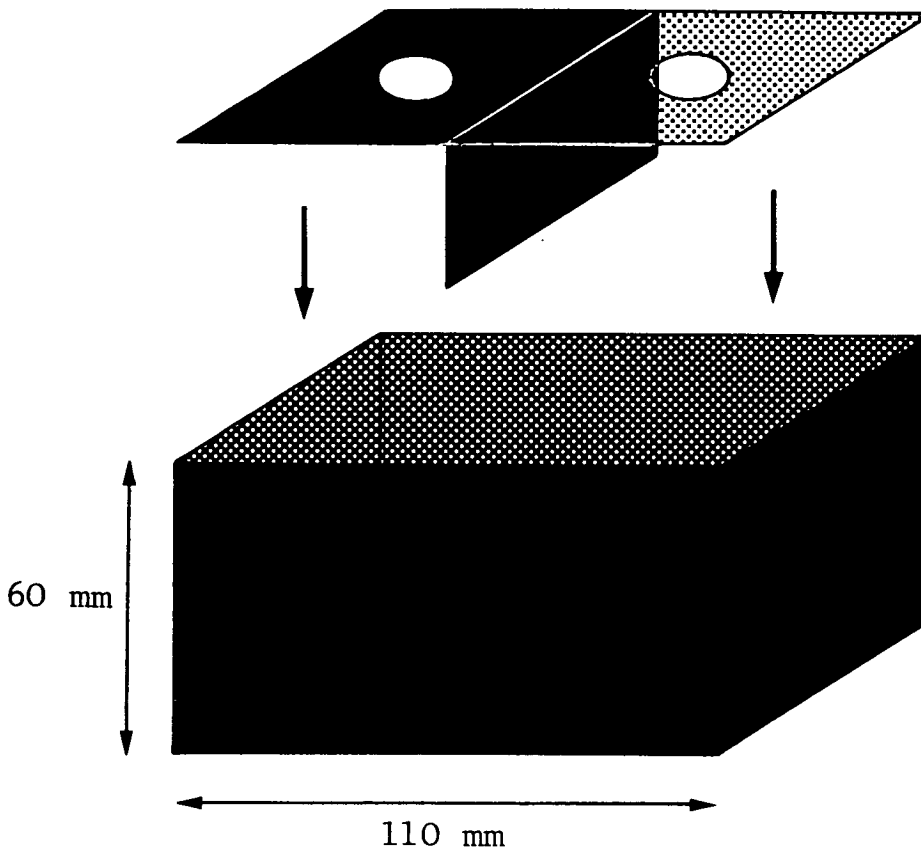
Figure 12. Light gradient apparatus used for larval photopreference in D. melanogaster.

11 x 11 x 6 cm glass staining dishes (figure 12) contained 20 mm of standard medium without living yeast. Bottom, sides and one half of the lid of the dishes were opaque to light. The other half of the lid absorbed 20 % of the incident light through a plexiglas filter so that the irradience at the surface of the medium below was 46 F.C.. A vertical opaque separation fixed at the lid increased the contrast between the two halves. The lids had two holes (∅ 1.5 cm) fitted with foam stoppers and they were fixed to the dishes with black tape.

3.2.4 - Method of egg collection

Experiments were initiated by eighty fertilized females coming from the base population, and egg collection was conducted as for D. simulans up to generation seventeen of selection. At this time it was decided to get rid of the microflora carried by the different selected lines (see under 2.4.4) to see whether different "microbial luggage" had previously had an effect on PSP. For this purpose the eggs of all lines were sterilized for three minutes in a solution of Karnovsky fixative pH 7.2. This treatment was applied at generation seventeen and eighteen, thereafter a modified method of egg collection was devised as follows to keep the eggs more free of microorganisms than previously.

Gravid females were allowed to lay eggs (light irradience of 9 F.C.) for fifteen hours on a paper towel moistened with yeast suspension in an egg laying pot (⌀ 35 mm, height 65 mm). The eggs were then thoroughly rinsed with distilled water through a 106 μm mesh size sieve and collected with a small paintbrush. One hundred eggs were carefully deposited along the midline of the dishes in order to allow a more accurate counting than in the previous experiments.

3.2.5 - Recording of PSP and selection

One hundred and twenty-five hours after laying the dishes of L- and DT-lines were opened in white light, while those of D- and LT-lines were opened in deep red light. As there were three dishes per selected line the same sample size of three hundred eggs at each generation was used as in the selection experiments using D. simulans. The numbers of pupae found in each half were counted and the t-test on mean percentage of pupae found in dark was used to compare different distributions. Fifty to sixty pupae were removed to ensure that twenty pairs could be used as parents of the next generation. From generation twenty-three onwards, twenty-five parental pairs were used instead of twenty in an attempt to lessen the effect of inbreeding. Pupae selected in the dark halves of the dishes always came from the long side (extreme dark) or very occasionally from the contiguous first ten milli-

metres of the short sides of the rectangular dark halves.
Very few pupae were found at the surface of the medium.
The selected adult pairs were mated at random and exposed
to the same environmental conditions as the pupae which
gave rise to them had been exposed.

3.2.6 - "Rewarded" and "traumatic" environmental
          conditions

        The lighting conditions to which the different
selected lines were exposed were as follows :
                1) Light preference "rewarded" line (L-
line) : constant light of an irradience of 90 F.C. from
the stage of newly formed pupae up to four or five days
after eclosion.
                2) Light preference "traumatic" lines (LT-
lines) : constant darkness until emergence, then 12 hour
dim light (9 F.C.)/ 12 hour dark cycle till four or five
days after eclosion.
                3) Dark preference "rewarded" lines (D-
lines) : same treatment as LT-lines.
                4) Dark preference "traumatic" lines (DT-
lines) : constant horizontal white light of an irradience
of 236 F.C., from the stage of newly formed pupae till
four or five days after eclosion.
L-, D1- and DT1-lines were selected for twenty-three to
twenty-nine generations. The selection of LT1-line was
stopped at generation nineteen because of a sudden un-

explained decline in viability. At generation twenty-four
a second LT-line (LT2-line) was set up from sixty-eight
residual pupae of L-line (removed from the light halves
of the dishes) and was successfully maintained up to
generation twenty-nine.

About twenty months after D1- and DT1-lines
were started, replicates of these lines were set up from
the base population and were run simultaneously for eight
generations (D2- and DT2-lines). PSPs of these two lines
are not directly comparable to those of D1- and DT1-lines
because the lids of the dishes were slightly modified in
order to start the selection with a mean PSP closer to
the middle of the scale of preference. The coefficient of
absorption of incident light of the light half of the lids
was 60 % (irradience of 23 F.C. at the surface of the
medium), instead of 20 %. This will be referred to as
design II.

3.2.7 - SubDT1-line kept in darkness for three generations

At generation 19 of selection fifty residual
dark preferring larvae were collected (when pupae) from
DT1-line to set up a subDT1-line whose flies were no
longer selected, but were still mated in pairs. Moreover,
these flies had no more traumatic treatment but were
instead treated like the D1-line's flies.

3.2.8 - SubD1-line kept in light for three generations

At generation 20 of selection, fifty residual dark preferring larvae were parallely collected (when pupae) from D1-line to set up a subD1-line, whose flies were also no longer selected but mated in pairs. The flies were exposed to permanent light similarly to those of the L-line.

3.2.9 - Lines kept in permanent light and permanent darkness (PL- and PD-lines)

Two samples of fifty females from the base population served to set up one PL-line and one PD-line in the same way as for D. simulans (see under 2.2.14). The PL-line was this time exposed to a light irradience of 236 F.C. (as DT-lines) and PSPs of both lines were assayed at generation 7, using design II (see 3.2.6).

3.2.10 - Control-line (C-line)

Twenty pairs of unselected flies issued from the base population were randomly mated in each generation to constitute a control line. This line was maintained for eight generations under a 12 hour light (50 F.C.)/12 hour dark cycle.

3.2.11 - <u>Chromosomal analysis of larval phototaxis</u>

This investigation was made possible by the use of a <u>D</u>. <u>melanogaster</u> stock carrying a balanced lethal system,kindly given to me by Dr. Brian Charlesworth (University of Sussex). Chromosomes II and III of this tester stock carried recessive lethal genes used as dominant markers. Chromosome II carried Curly (<u>Cy</u>) gene on one member of the pair and Plum (<u>Pm</u>) gene on its homologue. Chromosome III had Stubble (<u>Sb</u>) gene on one chromosome and Ultrabithorax (<u>Ubx</u>) on its homologue. Table 39 (p. 175) shows the mating plan used for a partial assay as described by Hirsch (1967), although the X chromosome carried no marker gene in my experiment. In this table X^L refers to an X chromosome coming from the L-line.

The same balancer stock was later used in order to carry out a chromosomal analysis between D2- and DT2-lines (3.3.6).

3.2.12 - <u>Production of highly inbred flies and estimation of isogenicity</u>

An investigation of cytoplasmic effects was attempted by using a long-inbred strain of <u>D</u>. <u>melanogaster</u>, kindly given to me by Dr. Trudy F.C. Mackay at the Institute of Animal Genetics of Edinburgh. This Samarkand stock

had first been inbred for many years by Dr. R. Middleton
at Birmingham University and then further inbred by full-
sib mating for fourty-seven generations in Edinburgh.

The isogenicity of these flies was tested by
estimation of the heritability of sternopleural bristle
number as measured by the linear regression of mean off-
spring value on mid-parent value. As Falconer (1981)
shows, the regression coefficient b is equal to the real-
ized heritability (h^2) as defined under 2.3.1. A test for
isogenicity was performed on IC-line (see below) one
generation before PSPs of the three inbred lines set up
were assayed, using design II (see 3.2.6).

3.2.13 - Mass mated inbred lines kept in permanent light
          and permanent darkness (IPL- and IPD-lines)

A first such an inbred line (IPL-line) was
exposed to a permanent light of an irradience of 236 F.C.
for seven generations. A second line (IPD-line) was kept
in total darkness for the same number of generations.
The flies were mass mated and twenty-five parental females
were allowed to lay eggs for fifteen hours in one-third
pint milk bottles to run each daughter generation. A
control line (IC-line) was treated the same way but
exposed to a 12 hour light (50 F.C.)/12 hour dark cycle.
Both at generations 4 and 7 one to two days old flies of

IPL- and IPD-lines were exposed to identical lighting conditions of 12 hour light (50 F.C.)/12 hour dark cycle. Eggs were then collected in each line from twenty-five females (when five to six days old) and PSPs of both lines were assayed simultaneously. Sex ratios of both light and dark preferring late larvae were estimated from the flies emerging from pupae found in both extreme light conditions in each line. PSP of IC-line was only assayed at generation 4.

At generation 4 and 7 reciprocal hybridizations were carried out between IPL- and IPD-lines in order to assess PSPs of both progenies as well as the sex ratios of both light and dark preferring late larvae.

3.2.14 - Inbred lines selected for dark PSP with "reward" and for dark PSP with "trauma" (ID- and IDT-lines

Twenty to thirty females issued from the above IC-line were mass mated for three generations (owing to lack of manpower) before one ID-line was set up to undergo exactly the same selection procedure and environmental conditions as the outbred D-lines. After two generations of this selection an additional IDT-line was set up from fifty-seven remaining dark preferring larvae from ID-line. This IDT-line was then treated as the outbred DT-lines. ID- and IDT-lines were always run simultaneously in the dishes.

3.2.15 - Other data collected related to PSP

Several other data were collected using methods identical to those used for D. simulans, such as PSPs of progeny from reciprocal hybridizations between divergent strains, egg-larval mortality, pupal mortality, fecundity and sex ratios of either light or dark preferring late third instar larvae. Egg-larval mortality was this time arbitrarily estimated to be approximated with about $\pm$ 5 % accuracy, assuming that the counting procedure of the eggs deposited (100 eggs $\pm$ 5 eggs) in the dishes was slightly improved in this second set of experiments.

3.2.16 - Recording of oviposition site preference (OSP)

The test vials shown in figure 3 (p. 51) were also used to assess OSP of D. melanogaster L-, D1- and DT1-lines. In order to test flies that had gone through identical lighting conditions prior to the test (= un-conditioned flies), twenty females from all lines to be tested were first allowed to lay eggs in light (irradience of 50 F.C.) for eight hours in bottles provided with fresh medium and living yeast. Pupae, then pairs of adults issued from these eggs were kept in vials under a 12 hour light (irradience of 50 F.C.)/12 hour dark cycle. Five days old females were tested at the end of the dark period. Proportions of eggs laid in dark were square rooted and arcsine transformed. Comparison between lines was done by

plotting 95 % confidence limits with the means of the
transformed data.

OSPs of progenies from the reciprocal hybridiz-
ations between L X D1-line were also assayed by using un-
conditioned flies as just described. Finally I measured
PSPs of larvae (issued from the base population) whose
parents' OSPs had previously been measured as above.

3.3 - Results

3.3.1 - Light preference rewarded line (L-line)

Figure 13 shows that the L-line responded well
to selection. For some reason the PSP of the parental
generation (0) was perhaps particularly low on the prefer-
ence scale (at 82 % in dark) comparatively with later
scores of both L- and D1-lines' PSPs. Since selection was
relaxed at generation 7 (like in D1- and DT1-lines), this
was taken into account when measurements of the responses
to selection and realized heritabilities were made. With
respect to this about ten generations of selection were
thus necessary for the L-line to reach a plateau, which
was noticeably more than the number of generations required
in D. simulans D-lines for a plateau to be reached.

By ignoring the result of generation 8 (presum-
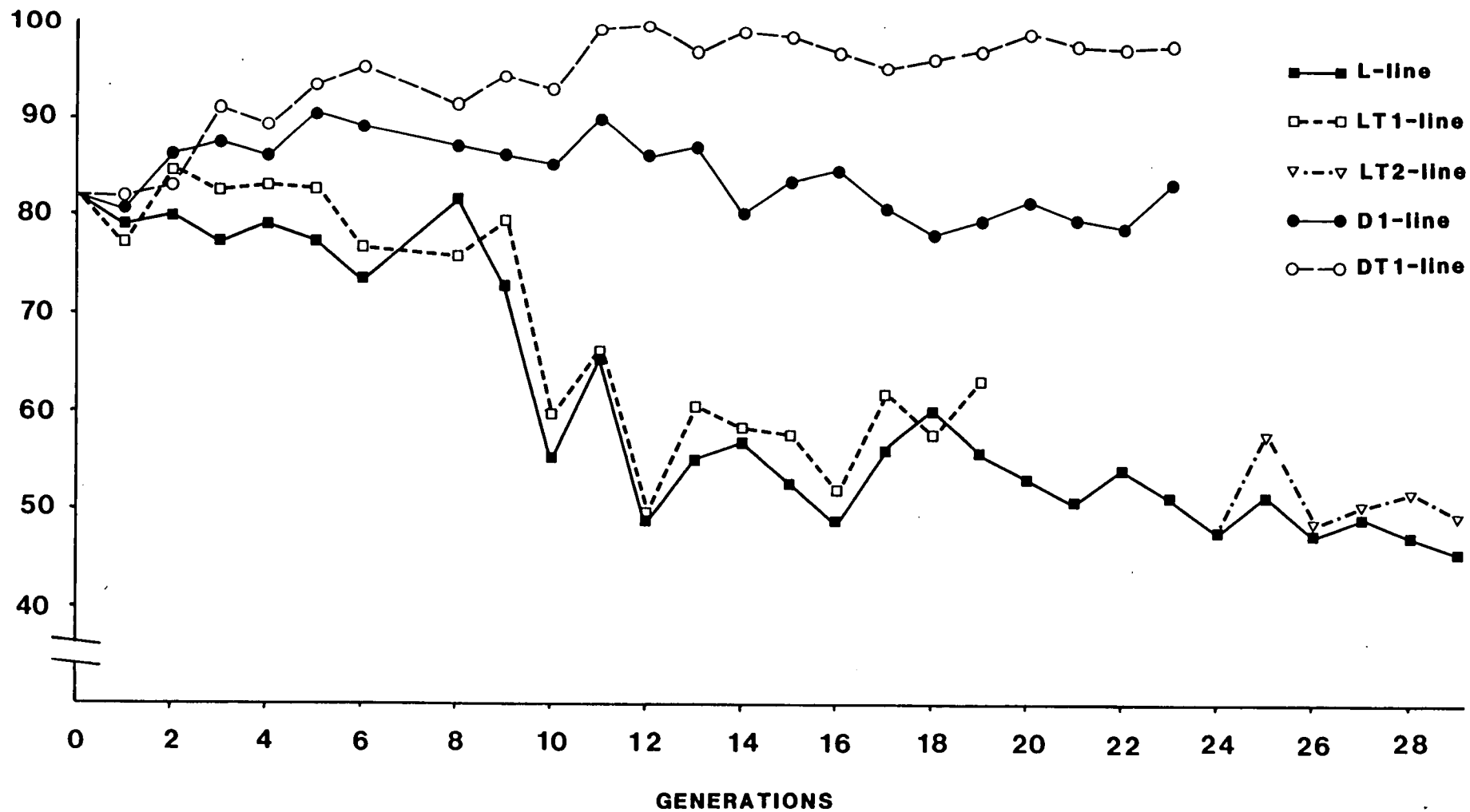
Figure 13. Mean percent of pupae in dark compartments plotted against generations of selection.

ably biased because of the relaxation of selection at the previous generation) a high cumulated mean response (33.5) was found after ten generations (table 40, p. 176). For the same generations the average response was R = 2.816. The correlation coefficient (r = 0.854) between R and progress of selection is significant at the .01 level when tested against ρ = 0 (correlation = 0). Although the response was strong, estimation of the realized heritability calculated over the same generations gives h^2 = 0.041 , which is relatively low but still significant at the .01 level when tested against β = 0 (slope = 0). This is not surprising when one considers that the selection differentials were high (table 40), and as Fuller and Thompson (1978) point out selection can indeed be very effective with low heritability if the selection differential is high as long as inbreeding is not too important.

By assuming that a plateau was reached after ten generations of selection (ignoring generations 7 and 8 in the data reported above), both response and realized heritability were measured in later generations. From generation 13 (11 in a more realistic figure) till generation 20 R =-0.487 and h^2 =-0.002 which confirms that selection was no longer effective after about ten generations.

3.3.2 - Light preference traumatic lines (LT-lines)

Figure 13 shows that PSP of the LT1-line remained very close to the L-line's PSP through the whole selection experiment. For the first ten generations of selection R = 2.694 and h^2 = 0.039 (both are significant at the .01 level), then from generation 13 till generation 19, R =-1.037 and h^2 =-0.006, all these values being very close to those of the L-line. Comparison of mean PSPs between L-line and LT1-line (table 41) shows that only three cases out of eighteen were significantly different at the .05 level.

The rewarded L - and traumatic LT2-lines set up at generation twenty-four of selection of the L-line were equally similar in PSP. Table 42 shows that none of the comparisons of mean PSP of the LT2-line with simultaneous PSP of the L-line were significantly different at the .05 level.

3.3.3 - Dark preference rewarded lines (D-lines)

PSP of the D1-line remained apparently unchanged as selection progressed (figure 13) and table 43 shows that the cumulated mean response of 3.9 after ten generations (computed in the same way as for the L-line) was almost nil. Still for the same generations the average response was R = 0.490 and the correlation coefficient

(r = 0.532) between R and progress of selection is just significant at the .05 level when tested against ρ = 0. It must, however, be stressed that the selection differentials were very low too (table 43), being with those of the DT1-line the lowest for all the selection experiments reported in this thesis.

Estimation of the realized heritability was consequently low (h^2 = 0.033) for the first ten generations of selection. Despite the fact that this is not significant (p $\sim$ .2) when tested against β = 0, it can be argued that an improved experimental design would be needed before drawing firm conclusions about response to selection for dark PSP. To further explore this possibility, the selection was started from a mean PSP closer to the mid-scale of preference, by making the light halves of the lids of the dishes more light absorbing as described under 3.2.6 (design II).

This D2-line was started with an initial PSP of 71.4 % in dark on the scale of preference. Figure 14 and table 44 show that apparently the change in design did allow a better response to selection for dark PSP to be expressed. For the first eight generations of selection, the cumulated mean response was 10.0 and R = 1.517 (r = 0.812, which is significant at the .01 level when tested against ρ = 0). Estimation of the realized heritability was also higher (h^2 = 0.073) compared to the h^2 of the

Figure 14. Mean percent of pupae in dark of D2- and DT2-lines plotted against generations of selection.

D1-line, which is now significant at the .05 level when tested against $\beta = 0$. Table 45 shows that after three generations of selection PSP of the D1-line differed significantly from PSP of the L-line at the .01 level. By and large the above results indicate a striking difference in selectability for PSP between D. simulans and D. melanogaster, which will be further discussed at the end of the present chapter.

3.3.4 - Dark preference traumatic lines (DT-lines)

Figure 13 shows that late larvae of the DT1-line apparently tended to select more and more dark pupation sites as selection coupled with traumatic treatment progressed. After ten generations of selection (computed in the same way as for the L- and D1-lines) the cumulated mean response was 16.8 (table 46) and the average response was R = 1.734. The coefficient of correlation (r = 0.935) is significant at the .01 level when tested against $\rho = 0$, even though the selection differentials (table 46) were lower than for the D1-line. Estimation of the realized heritability was $h^2 = 0.203$, which is significant at the .001 level when tested against $\beta = 0$. In order to make sure that the divergence observed between the D1-line's PSP and the DT1-line's PSP had not merely been due to genetic drift a replicate DT2-line was set up later (see below).

As with the DT-lines in $\underline{D}$. $\underline{simulans}$, the first
ten generations considered above can be divided into two
subseries of generations (generations 1 to 5 and 6 to 10).
The "half responses" measured this way become R_1 = 2.440
and R_2 = 1.014,respectively. This suggests a rather pro-
gressive pattern of divergence between D1- and DT1-lines
which can this time be seen also from the slopes of the
responses measured between successive generations (table
46).

Table 47 shows that DT1-line PSP tended to differ
significantly from D1-line PSP only after about eight
generations of selection. Between generations 11 and 23 of
selection, all mean PSPs simultaneously recorded were
different at the .02 level.

Figure 14 shows that DT2-line late larvae again
showed more dark PSP when compared to D2-line PSP. The
DT2-line responded faster to selection than did DT1-line in
the earlier design. Table 48 shows that after eight gener-
ations the cumulated mean response was 18.9 and the slopes
of the successive responses support again a progressive
pattern of PSP change. The average response was R = 2.593
and the correlation coefficient (r = 0.935) is significant
at the .01 level when tested against ρ = 0. The realized
heritability (h^2 = 0.163) is significant at the .001 level
when tested against β = 0.
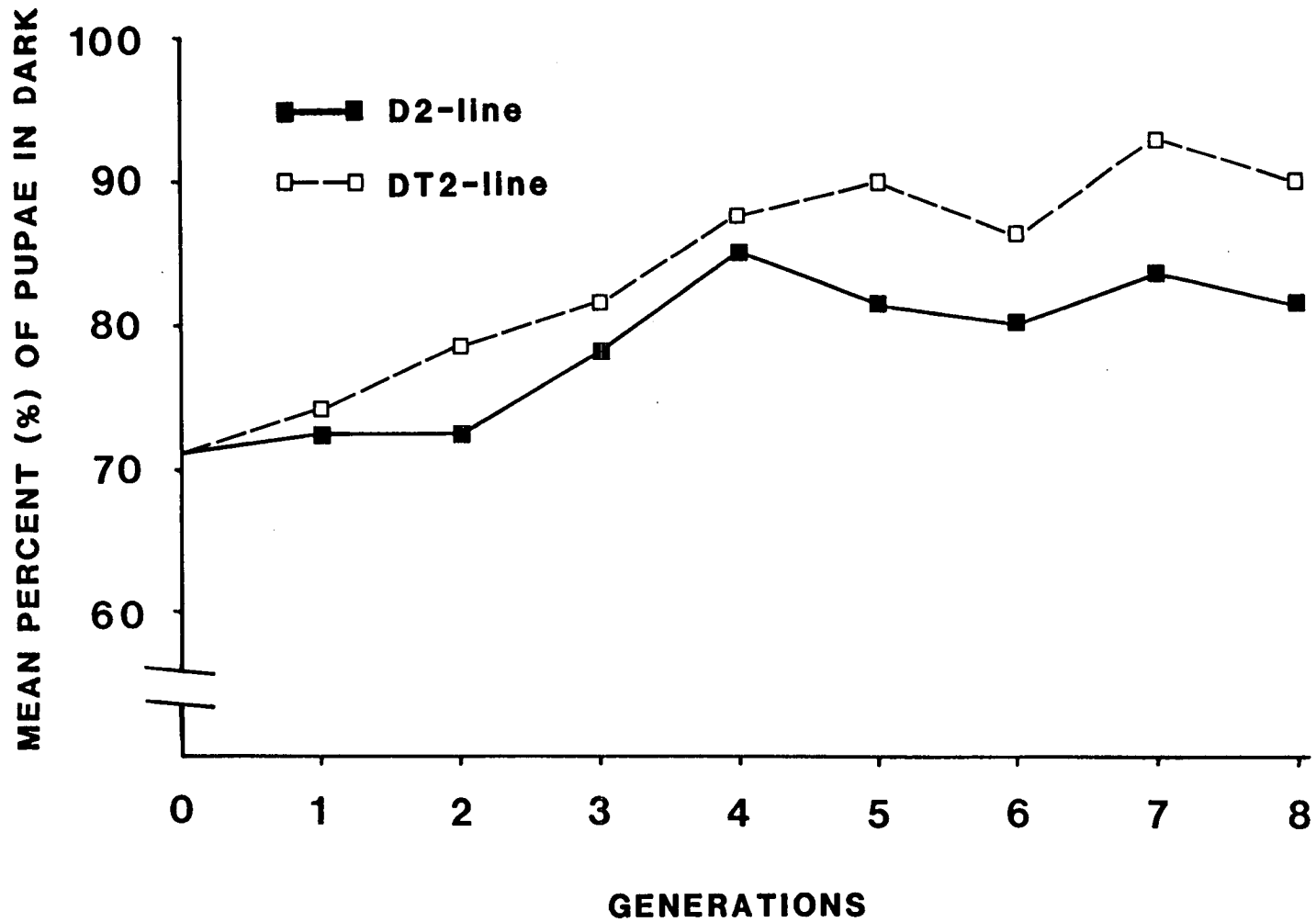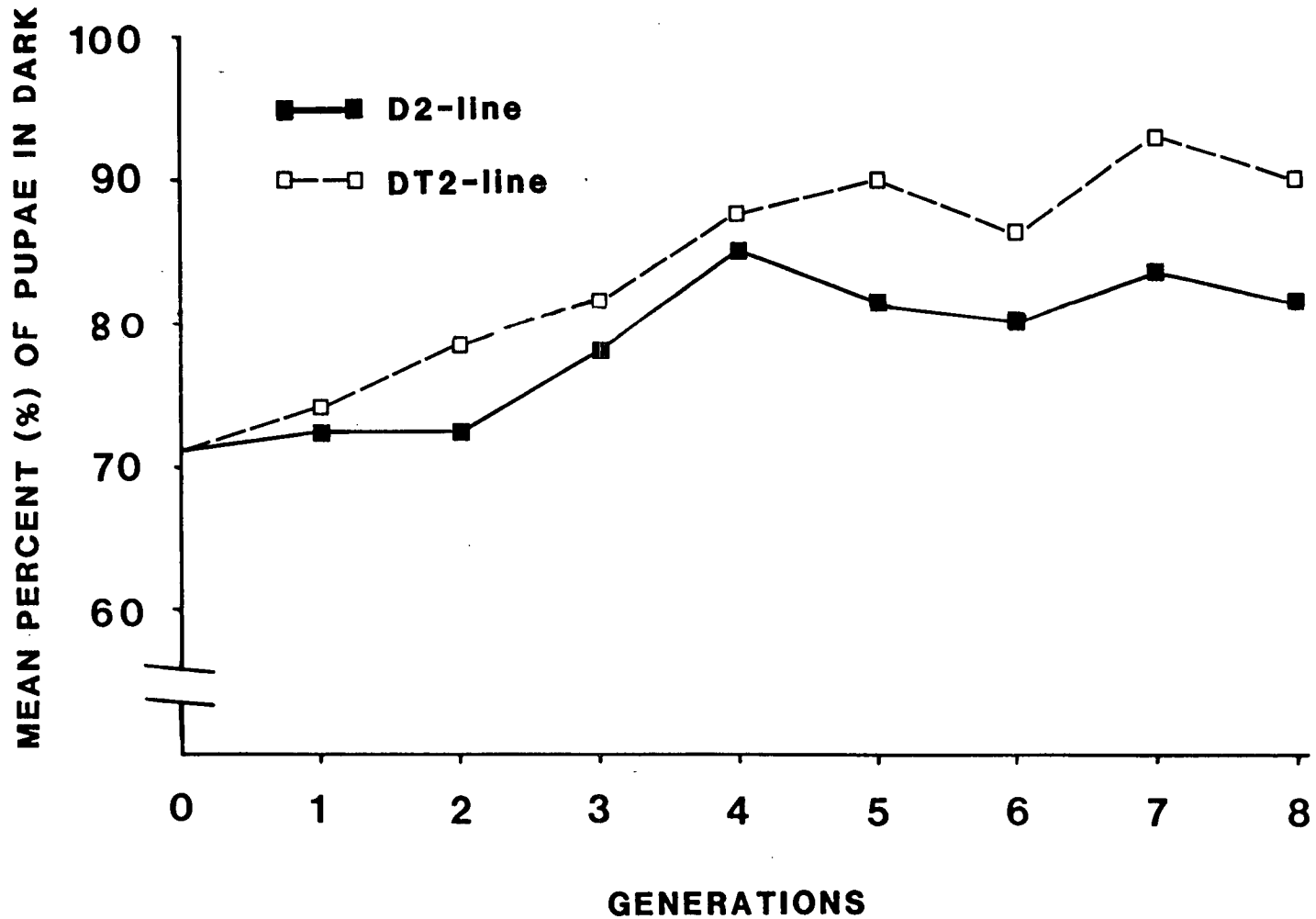
Figure 14. Mean percent of pupae in dark of D2- and DT2-lines plotted against generations of selection.

DT2-line PSPs were significantly different from D2-line PSPs after about six generations (table 49) therefore selection was stopped in both lines at generation 8.

3.3.5 - SubDT1-line kept in darkness and subD1-line kept in light

Table 50 shows that none of the mean subDT1-line PSPs compared between generations 20 and 22 were significantly different from each other. This stability strongly suggests that some genetic basis rather than an environmental cause was responsible for the separation between DT1- and D1-lines. If there was a direct conditioning (of the gametes or of the embryos exposed to light in DT-line parents) involved in the above divergence, its effect should have been reversed or cancelled in this experiment. Nevertheless, the long term stability of the factor(s) causing the observed difference was not investigated since this experiment was only carried on for three generations.

Furthermore, none of the mean subD1-line PSPs compared between generations 21 and 23 were significantly different from each other either (table 51). Environmental conditioning seems thus unlikely to be important here either. As expected, the relaxation of selection (although flies were still mated in pairs) was not accompanied by an important change in mean PSP after three generations since D1-line hardly responded to selection.

3.3.6 - Chromosomal analysis of D2- and DT2-lines

In order to investigate whether the difference observed between D- and DT-lines' PSPs was chromosomal, males of both D2- and DT2-lines were separately crossed with females from the balancer stock described under 3.2.11. The mating scheme used is shown in table 52 and it can be seen that the larvae of both distinct lines eventually tested carried the same cytoplasm stemming from the balancer females used at the start of the mating plan. In both lines four dishes were run in which PSPs of four hundred larvae to be tested were thus assessed. All eight dishes were run simultaneously. The mean PSP of the D2-line was 80.5 % (SE 1.1) in dark and that of the DT2-line was 82.2 % (SE 1.4). Comparison of the mean distributions using the t-test gives p < .3 ($|t|$ = 1.292, df = 6). This result suggests that the behavioural difference between the above lines, even though it is likely to have an hereditary basis as was demonstrated for D1- and DT1-lines, was not due to a chromosomal difference.

3.3.7 - Mass mated lines kept in permanent light and permanent darkness (PL- and PD-lines)

After six generations of exposure to permanent light, the mean PSP of the unselected PL-line was 72.7 % (SE 1.3) in dark and after the same number of generations

of exposure to permanent darkness PSP of the unselected
PD-line was 68.6 % (SE 1.1). Four dishes were run in each
line and the above scores are significantly different with
p ∿ .05 (|t| = 2.427, df = 6). By comparison with the PSP
of the base population (71.4 %,SE 2.5) it looks as if PL-
line PSP remained more stable than PD-line PSP but the
possibility of a genetic basis for this difference was not
investigated. It is, nonetheless, worth noticing that at
first sight the permanent light of an irradience of 236 F.C.
did not affect PSP of PL-line as did the trauma in DT-
selected lines. Besides, the apparent trend is not in con-
tradiction with the divergence observed between D- and DT-
lines, although in PL- and PD-lines differences in male
mating success or female fecundity were obviously not
controlled as in the selected lines.

3.3.8 - Control-line (C-line)

        Table 53 shows that the C-line's PSPs measured
at generations 1, 4 and 8 were all very similar. This
supports the view that the changes in PSP observed in the
selected lines were unlikely to be merely attributable to
inbreeding depression, and this probably had little in-
fluence on the selected trait.

3.3.9 - <u>PSPs of larvae run in the light gradient apparatus</u>
<u>used for D. simulans</u>

In order to investigate a possible design effect
of the different light gradients used, larvae of L-, D1-
and DT1-lines were run at generation 19 in the long grad-
ient apparatus used for selection of larval photoprefer-
ences in <u>D. simulans</u>. The values thus measured for PSP
(computed according to the formula given under 2.2.5) were
very similar to the values obtained using the square dish-
es. Mean PSP of the L-line was 57.1 % (SE 3.5) in dark,
that of the D1-line 72.1 % (SE 5.0) and that of the DT1-
line was 97.5 % (SE 0.05). This indicates that larval
activity had probably no effect on PSP since in the 60 cm
long boxes the distance eggs-extreme light conditions was
three times what it was in the dishes. One can then pre-
dict from this resemblance between results that the run-
ning of <u>D. simulans</u> in the dishes (with 20 % of absorbed
light in the light halves of the lids) should display a
mean PSP around 60 % in dark. This was broadly confirmed
by a test performed with the base population of <u>D. simulans</u>
(kept for about sixteen months in a population cage). Six
dishes were simultaneously run and a mean PSP of 66.5 %
(SE 3.3) in dark was observed, suggesting that a differ-
ence of about twenty percent in mean larval phototaxis dis-
tinguishes the two sibling species. Manning and Markow
(1981), using a slightly different design, found a differ-
ence of about twenty-four percent.

3.3.10 - <u>L-line X D1-line reciprocal hybridizations</u>

At generations 12, 18 and 22 of selection, reciprocal hybridizations were carried out between L-line and D1-line and the summarized results are presented in table 54. Progeny of the crosses were run for comparison at generation 19 in the long gradient apparatus used for <u>D. simulans</u>. The emerging picture seems to be that of PSP of progeny from reciprocal crosses being similar, as opposed to what was found in <u>D. simulans</u>. More precisely, table 55 shows that PSP of progeny from L-line females X D1-line males significantly differed from L-line PSP, but not from D1-line PSP. In three out of four cases, the quantitative difference indicates that progeny from L X L are noticeably more light preferring than progeny from all other crosses. Therefore, the results seem to support so far ordinary autosomal dominance of genes influencing dark PSP. This would then differ from the results of Manning and Markow (1981), which supported sex linkage (3.1.1).

3.3.11 - <u>Sex ratios of light and dark preferring progeny issued from L-line X D1-line reciprocal hybridizations</u>

Table 56 shows that among progeny issued from L-line females X D1-line males, females avoided extreme light pupation sites. The difference in sex ratio between extreme

light and extreme dark preferring larvae was significant at the .01 level at generation 19 and was close to the .05 level at generation 23 (x^2 = 8.133 and 3.658 respectively, with df = 1). This result would this time be compatible with an X-linkage of dominant gene(s) involved in the control of dark PSP, which would then be in contradiction with the results of hybridization described in the previous paragraph. Nevertheless, such a sex linkage pattern will be largely confirmed by later results and a possible explanation for its non-manifestation in the PSP results of progeny from the reciprocal hybridizations in tables 54 and 55 will be discussed at the end of this chapter.

3.3.12 - D1-line X DT1-line reciprocal hybridizations

Table 57 summarizes the PSPs of progenies issued from D1-line X DT1-line reciprocal hybridizations. It must be stressed that the interpretation of these results is difficult because quite a high egg-larval mortality was observed among some of the progenies, as was also the case with D. simulans (see 2.3.12). The stage at which this mortality occurred could not be examined as unhatched eggs were no longer easy to see when the dishes were opened for PSP assessment.

At generation 14 of selection about 94 % of the descendants from the DT1-line female X D1-line male cross

reached the pupal stage but only 69 % of the progeny
issued from the reciprocal cross did so. At generation 21,
the corresponding survival rates were 75 % and 63 %
respectively. Such high prepupal mortality was never
observed among progeny issued from the other crosses per-
formed, for which it very rarely exceeded 10 %. Since on
theoretical grounds hybridizing different strains is more
likely to increase general fitness through heterosis, the
lowered viability observed is hard to explain. Table 58
gives probability values for comparisons of PSPs without
taking the mortality into account. Therefore no difference
in PSPs between progeny from the reciprocal hybridizations
is reliably demonstrated by the data relative to the
pattern of transmission of the genetic change induced in the
DT1-line. Still the picture which emerges from table 57
would be that of extreme dark PSP being transmitted on a
paternal basis at generation 14, although this was not
significant at generation 21 (table 58). This point will
be more extensively discussed at the end of this chapter.

3.3.13 - Sex ratios of light preferring progeny issued from
the DT1-line female X D1-line male cross

At generation 14 of selection, 61.7 % of the
light preferring larvae issued from the DT1-line female
X D1-line male cross (the most successful cross) were
males. At generation 21 the corresponding proportion of

males was 58.8 % and in both cases the observed values are not significantly different from 50 %, but regrettably the samples were small here.

3.3.14 - LT2-line X D1-line reciprocal hybridizations

Table 59 summarizes the PSPs of progeny issued from the reciprocal hybridizations between the LT2-line and the D1-line. The purpose of this cross was to examine whether any difference in progeny PSP would be observed by using LT2-line mothers instead of L-line mothers. Tables 59 and 60 can then be compared with tables 54 and 55. The interesting point is that the results in tables 59 and 60 tend to support this time an X-linked pattern of transmission of dominant genes influencing dark PSP, whereas this was clearly not the case in results in tables 54 and 55, which even suggested a weak trend in the opposite direction. The t-test between PSPs of progeny from the reciprocal hybridizations in table 59 gives probability value p close to .05 ($|t| = 2.638$, df = 4). Again a possible explanation accounting for this discrepancy will be presented at the end of this chapter, assuming that the main difference in procedure between the two experiments is that L-line flies were descendants from flies exposed for several generations to light, while LT2-line flies were descendants from flies which experienced no light as pupae and young adults for five generations (see 3.2.6).

3.3.15 - <u>Sex ratios of light and dark preferring progeny</u>
            <u>issued from LT2-line X D1-line reciprocal</u>
            <u>hybridizations</u>

Tables 61 and 62 show that results from the sex ratios of both light and dark preferring progeny from LT2 X D1 reciprocal hybridizations further support the trend towards an X-linked effect with dark preference dominant but this was hardly significant (p $\sim$ .06 in extreme dark). Comparison between the sex ratios found among both extreme light and extreme dark preferring progeny issued from LT2 females X D1 males (tested by using a contingency table) gives x^2 = 3.177 (df = 1), while x^2 = 0.365 (df = 1) for progeny from the reciprocal cross (p < .1 and p < .7 , respectively). Here again the sample size was regrettably small.

In order to dissect genetically PSP, as a complex trait likely to be polygenically controlled, a straight-forward chromosomal analysis was carried out to estimate the role played by chromosomes II and III in the behaviour investigated.

3.3.16 - <u>Chromosomal analysis of larval phototaxis : mating</u>
            <u>regime and results</u>

Progeny from thirty females from the balancer stock were assayed in four dishes before crosses were under-

taken and the mean PSP of these multiply-marked flies was
85.5 % (SE 2.0) in dark. I decided to use tester males in
order to better control the origin of the cytoplasm stem-
ming from the L-line tested. For this purpose thirty
tester females carrying Cy, Pm, Sb and Ubx genes were
firstly mated at random with thirty L-line males coming
from generation 30 of selection (table 39,p. 175). Progeny
were run in four dishes and the mean PSP was 86.0 % (SE
1.2) in dark, which seems to indicate that the genes res-
ponsible for dark PSP in the tester stock females were
strongly dominant over their alleles in L-line males.

Sex ratios of both light and dark preferring
progeny issued from the above cross turned out to be of
some interest for they can legitimately be compared with
the results obtained for progeny from the D1-line female
X L-line male cross (table 56). While no biased sex ratio
was found among progeny from the latter cross using D-line
mothers, strongly biased sex ratios were observed among
progeny from the former cross using tester females (which
showed high mean PSP in dark too as just mentioned). Table
63 indicates that females strongly avoided extreme light
pupation sites and sought rather extreme dark sites in-
stead. The difference between the sex ratios found among
extreme light and extreme dark preferring progeny (tested
by using a contingency table) is significant at the .01
level (x^2 = 8.563, df = 1). The results suggest that the
Y chromosome of the L-line had an effect on the action of

the X-linked genes of the balancer females, as the X-linked dominant effect seems to be lessened in the hemizygotes.

All females from the progeny issued from the previous cross (tester females X L-line males) were discarded as well as three male genotypes as shown in table 39 (p. 175). Males carrying both Cy and Sb were then backcrossed with L-line females. Genotype frequencies of the progeny were used to analyse the genetic basis of larval phototaxis and results are presented in table 64. Genotype frequencies were computed from phenotype frequencies of three hundred and thirty-one males, and three hundred and fifty-six females. Significant departure from an expected 25 % frequency of each genotype was first calculated in both light and dark, and for each sex separately. Egg-larval mortality was about ten percent, and mortality at the pupal stage was about two percent in light and four percent in dark.

The results in table 64 do not clearly establish whether a deviation in frequency from 25 % was more due to the presence of a L-line chromosome than to the action of a mutant gene (Cy or Sb) determining more dark PSP. Nevertheless, the mean PSP of the tester stock at 85.5 % (SE 2.0) in dark suggests that the mutant genes had only a weak effect towards darker preferences, when compared with the mean PSP in dark of the base population (at 82.2 %, SE 2.5, in

dark) from which the tested L-line stemmed. Table 65 indicates that in males both chromosomes II and III carried genes determining positive phototaxis in L-line. Interestingly the situation is apparently different in females, for which only chromosome III seems to be involved in PSP. In both males and females table 64 suggests that chromosome III from the tester stock (carrying Sb) determined rather dark PSP, but again this effect might have been stronger in females than males ($p < .01$, and $p \sim .18$, respectively). This view is better supported by the statistics reported in table 65 where the different phenotype frequencies are more directly compared.

It must be pointed out that table 39 indicates a genotypic difference between males and females assessed, due to the origin of their X chromosomes. In F2 females there is an X chromosome from the tester stock which is not present in F2 males. According to an X-linkage pattern of transmission of dominant genes controlling dark PSP, this situation is expected to cause slightly more female larvae than male larvae to pupate in the dark, and results in table 66 tend to support such a trend. Comparison between the sex ratios found among both extreme light and extreme dark preferring progeny (tested by using a contingency table) gives $p < .1$ ($x^2 = 3.310$, df = 1).

3.3.17 - <u>Comparison between the fecundities of L-line and</u>
        <u>D1-line</u>

At generation 18 of selection the mean number of
eggs laid per partly starved female per twenty-four hours
(see under 3.2.15 and 2.2.19) was 37.8 (SE 3.0) for the L-
line and that of the D1-line was 54.3 (SE 4.4). At gener-
ation 28 the values had fallen to 21.1 (SE 1.8) for the
former and 34.3 (SE 1.6) for the latter. This difference
(significant at the .05 level in both cases, using the t-
test) is in accordance with the genetic architecture of
PSP apparently suggested from results of selection. If the
asymmetry in response to selection for light and dark PSP
(3.3.1 and 3.3.3) implied that the characters selected, or
some other characters correlated with them, were components
of natural fitness, selection towards decreased fitness
(as in the L-line) would be expected to give a faster
response than selection towards increased fitness (as in
the D1-line).

3.3.18 - <u>Comparison between the fecundities of D1-line and</u>
        <u>DT1-line</u>

In order to examine whether the differences
observed in PSPs between D1-line and DT1-line could have
been due to differences in fecundities, these were record-
ed at generations 18 and 28 of selection. At generation 18

the t-test on mean number of eggs laid per female per twenty-four hours gives p < .2 ($|t|$ = 1.472, df = 34) and at generation 28 p < .5 ($|t|$ = 0.921, df = 43). Still this result does not rule out the possibility that a larger difference could have been recorded over the generations where the DT1-line separated from the D1-line, at the beginning of the selection experiment (figure 13).

3.3.19 - Mortality of L-, LT1-, LT2-, D1-, D2-, DT1- and DT2-lines

Egg-larval mortality was low and remained at about the same level as selection progressed. In a similar way, Sharp (1982) found that after twenty generations of full-sib mating in D. melanogaster, egg-to-adult viability showed surprisingly little inbreeding depression. Mortality scores were found to be similar to those observed in D. simulans and did not exceed 8.5 % (table 67). Again the values reported in table 67 may have been slight overestimates since some eggs may have been damaged during the egg collection procedure.

As in D. simulans all traumatic lines tended to be slightly less viable (but not significantly) and it must be kept in mind that larvae of all lines experienced identical environmental conditions. Similar results were obtained for the mean mortality rates which occurred at the pupal stage (table 68). This latter mortality was even

lower than the larval mortality in all lines studied and therefore there was no indication that strong intra-line selection was operating through differential pupal survival.

3.3.20 - <u>Sex ratios of L-, LT1-, D1-, D2-, DT1- and DT2-
         flies emerging from selected pupae</u>

Regrettably sex ratios of the adult flies emerging from selected pupae were only recorded up to the fifth to seventh generations of selection. Nonetheless, the results shown in table 69 are very intriguing. Selected pupae of both DT-lines showed sex ratios strongly biased towards females, whereas those of both D-lines were consistent in showing no departure from 1:1. At first sight then it looks as if in DT-lines some X-linked dominant alleles influencing dark PSP might have been somehow more frequent or more active than in D-lines, and this point will also be further discussed at the end of this chapter.

Since the results from the chromosomal analysis between D2- and DT2-line (3.3.6) did not indicate that the PSP difference observed was merely due to a chromosomal difference, it became necessary to test for any cytoplasmic or environmental effect on PSP maybe induced by the traumatic treatment in DT-lines.

One possibility was therefore to repeat the

selection procedure applied to outbred flies with highly inbred flies to test for any extranuclear effect. Flies made isogenic for nuclear genes were then used to set up several unselected and selected lines, exposed to various lighting conditions. It was hoped that assays of PSPs of descendants from these lines might be informative on the possible origin of the behavioural difference observed between D- and DT-lines.

3.3.21 - Control for isogenicity of the inbred stock used

Sternopleural bristle number was assayed in both members of sixty-seven parental pairs and then in six progeny (three males and three females) from each of the sixty-seven pairs. Isogenicity was tested by estimation of the realized heritability of this character (see 3.2.12) and the regression coefficient b = 0.0196, which is not significantly different from zero. When tested against $\beta = 0$, the probability value p is < .9 ($|t|$ = 0.271, df = 65). This gives some evidence that the control-line (IC-line, see 3.2.13) tested this way had been rendered isogenic through long-term inbreeding. Since the unselected IL- and ID-lines were treated like the IC-line except for the lighting conditions, it can be assumed that they were equally highly isogenic for nuclear genes.

3.3.22 - PSPs of IC-, IPL- and IPD- mass mated lines

Table 70 summarizes the results obtained with IC-, IPL- and IPD- mass mated inbred lines. Although there was a repeated trend for IPL-line to show slightly more dark PSP than IPD-line, the difference was not significant. Comparison of the sex ratios of both light and dark preferring larvae between the two lines indicated no significant difference either.

At generations 4 and 7 reciprocal hybridizations were carried out between IPL- and IPD-lines and results in table 70 show that there were no PSP differences nor sex ratio differences between progeny of reciprocal hybridizations. The results therefore indicate that with these isogenic flies there were no direct effects on parents or progeny of either constant light or darkness as was apparently the case when outbred PL- and PD-lines were treated in the same way (3.3.7).

In order to investigate more directly the possibility of an effect of the light trauma as it was applied to the outbred DT-flies, an isogenic IDT-line (selected and traumatic) was set up and assayed for PSP, as well as an isogenic ID-line (selected and rewarded).

3.3.23 - PSPs of ID- and IDT- selected lines

As described under 3.2.14 the ID-line was set up from the IC-line (see above) and the IDT-line was derived from the ID-line after two generations. ID-line was then selected for eight generations while IDT-line was selected for six generations only. Although this stage was the point at which PSPs of D2- and DT2- outbred flies began to diverge significantly, no divergence at all was found between ID- and IDT-inbred lines. Table 71 gives the mean PSPs of both lines for the six generations considered and scores are compared using the t-test.

The second experiment using inbred flies apparently also failed to show the effect observed with outbred flies. I shall still discuss later a possible interpretation of these negative results as it can be argued on theoretical grounds that the direct comparison between the outbred and inbred lines used must be made with caution.

3.3.24 - Oviposition site preferences (OSPs) of L-, D1- and DT1-lines' flies

At generation 18 of selection, oviposition site preferences (OSPs) of L-line and D1-line unconditioned flies were compared. OSPs were assayed and computed as described under 3.2.16 and table 72 shows that the 95 %

confidence limits plotted with the means of the transform-
ed data do not overlap.

This experiment was repeated at generation 28
where for practical reasons a different source of light had
to be used to illuminate the test vials, providing an
irradience of 269 F.C. instead of 213 F.C.. The consequence
of this was that females of both lines laid more eggs in
the dark halves of the vials, showing more avoidance of the
light halves. The results show that L-line and D-line OSPs
were this time not significantly different. This may have
been partly a consequence of the fact that in this exper-
iment few eggs were laid in light by females of either
line.

Taken together the above data are still likely
to indicate a genetic correlation between OSP and PSP,
similar to that found in D. simulans. In order to show this
less equivocally, unconditioned progenies (treated as just
described) issued from L-line X D1-line reciprocal hybrid-
izations were tested in the same way at generation 19
(tables 72 and 73).

Measurements of PSP of the progeny issued from
the reciprocally hybridized flies again supported the
conclusion that there was some genetic correlation between
OSP and PSP. Although there is no significant difference
between OSPs of the progeny from the reciprocal hybridiz-

ations, OSP of progeny from D1-line female X L-line male cross still differ from L-line OSP but not from D1-line OSP. Taken at face values the data actually suggest almost complete dominance of genes influencing dark OSP. Therefore, the results seem to be consistent with the postulate that genes controlling dark PSP could act through a pleiotropic effect on OSP, since the same trend of genes influencing dark preferences being dominant was found both in preadult and adult behaviours.

At generation 28 of selection, unconditioned DT1-line females were tested for OSP and the results are shown in table 72. This experiment was unfortunately also performed under too strong incident light (269 F.C.), resulting again in dark OSPs of the DT1-line (as those of the D1-line) not significantly different from those of the L-line. Still,it looks as if the flies of the DT1-line did not oviposit in darker areas (table 72) than the flies of the D1-line, which would parallel what was observed in D. simulans, where the light trauma was found to have no effect on OSP either.

3.3.25 - PSPs of larvae issued from the base population, whose parents' OSPs were known

A last experiment aimed at investigating the possibility of a PSP-OSP genetic correlation was initiated

by a few flies of the base population allowed to lay eggs
in bottles provided with fresh medium and living yeast.

Pupae of the progeny of these flies were put in
pairs in vials and females were tested for their OSP when
seven days old (irradience of 269 F.C.). Out of thirty
females tested, eight were chosen for their most light OSP
and seven for their most dark OSP (table 74).

Compared with the selected lines there was a
relatively high proportion of eggs laid in light by these
flies which remains difficult to explain unless this was
due to age since seven days old females were used here
instead of five days old in all previous tests conducted
under the same conditions.

The two groups were then allowed to lay eggs
under identical conditions of light (9 F.C.) and these
were deposited in the dishes in order to assay PSPs of
both groups. Table 74 shows that the mean PSP of the
larvae of each group was significantly different at the
.01 level, which confirms the existence of the postulated
genetic correlation between the larval and adult photo-
tactic traits investigated.

3.4 - Discussion

3.4.1 - Genetic architecture of larval phototaxis in D. melanogaster

The intriguing finding about the genetic architecture underlying PSP in D. melanogaster is its great contrast with the corresponding architecture found in D. simulans. In D. melanogaster, results from bidirectional selection suggested that there is only limited amount of additive genetic variation for dark PSP, implying that this trait is likely to be this time under stronger directional natural selection. By considering again the main consequence of Fisher's (1930) "fundamental theorem of natural selection", characters exhibiting little additive genetic variation are expected to be close to fitness and to suffer greatly from inbreeding depression.

Asymmetry in response to artificial selection for PSP was indeed observed again in D. melanogaster, but with selection for light PSP being now easily feasible, as opposed to the results for D. simulans. Hybridizations of divergent selected lines indicated this time a high degree of dominance of genes (or some of them) determining dark PSP over their alleles determining more light PSP. It must still be pointed out that, just as for the previous experiments with D. simulans, the way in which the realized heritability of PSP was estimated in L-, D- and DT-lines is

not entirely satisfactory as the selection differentials
could not be appropriately weighted from the data. As the
same number of offspring of the selected parents were
measured in all up- and down-lines, no "effective" select-
ion differentials could then be weighted in order to take
account of a good part of the effects of natural selection.
Natural selection was likely to be operative through
differences of fertility as some results relative to L-
and D-lines indicated (3.3.17). It was observed that the
response to selection for light PSP in $\underline{D}$. $\underline{melanogaster}$ was
substantially weaker than the response for dark PSP record-
ed in $\underline{D}$. $\underline{simulans}$, even though the selection differentials
were higher in the former case. The best between line
comparison of the proportion of the total variance that is
attributable to the average effects of genes involved in
PSP is therefore provided by the heritabilities, bearing
in mind that these were not totally adequate estimates
here. In $\underline{D}$. $\underline{simulans}$ h^2 of the L-lines = 0.001 and 0.005
respectively, whereas in $\underline{D}$. $\underline{melanogaster}$ h^2 of the L-line
= 0.041. In contrast h^2 of the D-lines in $\underline{D}$. $\underline{simulans}$ =
0.177 and 0.183, whereas that of the D1-line in $\underline{D}$. $\underline{melano}$-
$\underline{gaster}$ = 0.033. The absence of scale effect of the two
different designs used to assay PSP was demonstrated by
running the two species in both types of light gradient
apparatus since scores were very consistent between
designs (3.3.9).

As just mentioned, the comparison of fecundities

of L- and D-lines in <u>D</u>. <u>melanogaster</u> corroborated the
possible higher fitness of D-line flies relatively to L-
line flies so that the genetic architecture emerging from
my results is well in line with the results reported by
Manning and Markow (1981). Although the involvement of
the X chromosome in PSP was suggested by several results,
the chromosomal analysis indicated that chromosomes II and
III also influence the trait under study. Therefore PSP
appears to be polygenically controlled - a similar situa-
tion to that found by Markow (1975b) for adult phototaxis.
Since at least three of the four chromosomes contribute
to control PSP, the relative magnitude of each of these
components needs to be investigated, as it could cast
some light on the remarkably high level of PSP variation
observed between individuals. It is indeed worth noting
that in each trial of PSP (i.e. in each dish) several
pupae were invariably found both in extreme light and in
extreme dark, in all lines studied.

With respect to the way of reasoning outlined
above which takes into account general fitness it is then
surprising that no effect of inbreeding on PSP was found.
No change in PSP was observed in the control-line, and
neither the multiply-marked balancer stock nor the highly
inbred I-lines manifested any increased mean preference
for light, as would be predicted from a traditional model
of dominance related to fitness. Furthermore, the egg-to-
adult viability was not significantly different between

L- and D-lines either. Although the values found for the
realized heritabilities of L- and D2-lines confirmed the
presence of some additive genetic variance for PSP in the
base population of D. melanogaster I used, there still
remains a non-additive genetic component of the phenotypic
variance to be considered. This is due to the dominance
deviation of some of the genes involved in PSP as well as
to the probable epistatic interaction between at least
some of the genes involved. Nonetheless, the persistence
of extreme light- and extreme dark- preferring late larvae
in both L- and D-lines after more than twenty generations
of directional selection certainly remains hard to ex-
plain, even if one considers that powerful environmental
effects can sometimes "flip" development.

3.4.2 - Correlation between preadult and adult preferences

The recording of OSP in L- and D1-lines strong-
ly indicated that larvae with dark PSP tended to produce
offspring which had darker OSP than did larvae with light
PSP. The results therefore confirm the existence of a
genetic correlation between PSP and OSP, as already
suggested from the results of the previous chapter. I
shall further comment in chapter 5 on the observation
that in both D. simulans and D. melanogaster the light
induced shift in PSP was apparently not paralleled by a
shift in the same direction in OSP.

The demonstration of a genetic correlation between PSP and OSP provides some factual evidence in support of the view that some degree of habitat loyalty with respect to light conditions may exist in nature in Drosophila. Moreover, the results reported so far suggest that in D. simulans the most light preferring individuals are likely to be the fittest, while in D. melanogaster the most dark preferring individuals are expected to be the fittest. This finding might indeed point to the possibility of a mechanism for the splitting of a single population into two sexually isolated populations, as it might have operated among some common ancestors of the two present sibling species. Such a mechanism would thus fulfil the preliminary condition required for sympatric speciation to become plausible (as mentioned in 1.2), for which the fitness of different genotypes selecting different habitats should vary between these habitats. Further investigation on the possibility that the two species studied in the present context really exploit different microniches with respect to light conditions in the wild is still needed, although some evidence for its occurrence was presented in 1.8.3.

Since OSP assessments were only carried out at generations 18 and 28 of selection using slightly different procedures, it is difficult to know if this trait was subject to linear inbreeding depression. Nonetheless, the unusually positive mean value of photopreference scored

in adult flies issued from the base population mentioned
under 3.3.25 suggests that OSP was unlikely to be altered
through inbreeding since these flies were in fact sub-
stantially more outbred than those tested at generations
18 and 28. The relatively marked difference observed in
PSP between the two groups of animals tested in the last
experiment on PSP suggests again that at least some of
the genes involved in the trait studied had large effects.

3.4.3 - Effect(s) of the traumatic environmental
treatments

Table 75 summarizes the effects on PSP that
either rewarding or traumatic environmental treatment had
in the main lines studied. As pointed out about D. simu-
lans,it appears that with outbred selected flies only
the trauma associated with light affected PSP (in DT-
lines),supporting again the possibility that the induced
change in PSP may have occurred through some photoactiv-
ation mechanism. The evidence for a genetic basis under-
lying the difference between D1- and DT1-line was given
by the results relative to subD1- and subDT1-lines (3.3.5).
It was still mentioned that the absence of light might
have altered the PSP of the outbred mass mated flies (PD-
line) in a consistent way but a genetic basis for this
difference was not demonstrated.

The separation of DT-lines from D-lines seemed

to require more generations in this experiment than was necessary in the experiment using D. simulans, although this might be partly explained by a mere difference in selection conditions. One must remember for instance that the selection differentials of all D- and DT-lines were substantially lower in D. melanogaster than in D. simulans. This point was apparently supported by the observation that the separation of DT2-line from D2-line took place faster than the separation of DT1-line from D1-line, as was expected from the improvement made to the design of the light gradients. It is also possible that the above discrepancy in the rate of divergence between the two species was more simply due to a greater "sensitivity" of the long box gradients used in D. simulans, which presented the larvae with a more regular gradient of light intensities.

In D. melanogaster the separation of DT-lines from D-lines was nonetheless more progressive than in D. simulans, so that it looks as if there was a cumulative effect of the traumatic treatment over generations. The change in DT-line PSP relative to D-line PSP again deserves special attention as at first sight no obvious explanation can account for it in terms of conventional selection. Unless it is supposed that light might have directly induced some genetic disorder like hybrid dysgenesis which causes sex ratio distortion, not many possibilities can be envisaged at present. It was shown that

indeed differences in either fecundity or egg-to-adult mortality were unlikely to account for the results. If one of these (or both) were responsible for the findings, it would imply that the light trauma would have somehow favoured the fittest flies, and that these happened to be carrying a good proportion of genes influencing dark PSP.

There is no a priori reason to expect a difference in egg-larval mortality as these were exposed at each generation to identical conditions in all lines until artificial selection was performed. Pupal mortality was shown to be too low to deserve more consideration. The remaining possibility is then that the relative egg-laying rates of different classes of individuals were affected by the trauma, although this was hopefully lessened by exposing the selected mothers of all lines to the same lighting conditions for nine hours before eggs were collected. Nevertheless, a long lasting conditioning due to the trauma cannot be ruled out. It must still be pointed out that if light had such an effect it can be argued that this should have affected PL-line as well, as flies of this line were exposed to the same light intensity as DT-line flies. However, at generation 7 PSP of the PL-line was at about the same level as that of the base population. Although the light intensity experienced by L-line pupae and young adults was lower than that in DT-lines, the response to selection (toward lighter PSP) of L-line was apparently not noticeably counteracted by a light induced

effect either.

In D. melanogaster the light trauma thus tended to enhance dark PSP, and interestingly independant results sometimes suggested that progeny whose female parent came from a line exposed to light tended to have more dark PSP than progeny whose female parent came from a line kept in dark. Such a view was, however, not supported by the results of the reciprocal crosses between D1- and DT1-lines, although it was stressed in 3.3.12 that these results are little reliable because of high prepupal mortality.

Additionally, it was suggested that the above dark PSP enhancement tended to be more marked in females too, as if the degree of dominance of some X-linked allele(s) involved in dark PSP was somehow increased through light inducement. The results which might support such possibility can be summarized as follows :

1) DT-line larvae had more dark PSP than D-line larvae and showed a sex ratio biased towards females in dark, in contrast with what was found for D-lines.

2) Although the results indicated that the X chromosome was involved in PSP, reciprocal hybridizations between L- and D-lines failed to support this observation. Following the way of reasoning outlined above,progeny from L-line females X D-line males might

have had enhanced dark PSP compared with progeny from the
reciprocal cross (tables 54 and 55), even though the re-
verse trend is expected from an X-linked dominant basis.
The results did suggest such trend which was significant
when using the long box gradient apparatus.

3) In contrast with progeny from the above
cross (L-line mothers X D1-line fathers), progeny from
LT2-line mothers X D1-line fathers would then be expected
to show the opposite trend, since LT2-line experienced no
light. Indeed the results in tables 59 and 60 tended to
support this time an X-linked pattern of transmission of
dominant genes influencing dark PSP.

4) Dominant genes influencing dark PSP were
presumably equally selected for in both D - and DT-lines,
therefore no sex ratio distortion would be expected among
progeny from the reciprocal crosses between these lines.
Nevertheless, there was a trend (not significant, but from
a small sample) for sex ratio to be biased towards males
in light among progeny from DT1-line females (which exper-
ienced light) X D1-line males (3.3.13).

The above results thus point to the possibility
that females might have been more susceptible to the light
trauma than males. Since the chromosomal analysis carried
out between D2- and DT2-lines (3.3.6) indicated that the
PSP difference between these lines was not just chromosom-
al, one workable hypothesis would again take into account
a cytoplasmic effect, as already pointed out in the dis-

cussion of the results obtained with <u>D</u>. <u>simulans</u>. However, such view was not supported by the results obtained with isogenic lines, although it can be argued that caution is necessary in comparing the data relative to inbred flies with those relative to outbred flies on the following ground. In highly inbred lines the alleles involved in PSP which became fixed through inbreeding were likely to be those related to high fitness, therefore probably corresponding to the dominant ones as argued earlier. If the light inducement ultimately tended to render recessive genes more dominant (as will be discussed in chapter 5), highly inbred flies might consequently be expected to be less responsive to the effect of the trauma than outbred flies which are more polymorphic at the loci under study.

In order to see whether similar traumatic effects could operate in other contexts, I decided to perform another set of experiments consistent with the selection pattern shown in figure 1 but using chemical compounds as environmental cues to assess early preferences, and these experiments are described in the next chapter.

Table 39

Mating plan for partial assay (from Hirsch, 1967). See text for the symbols used.

Table 40

Cumulated mean responses to selection of L-line calculated over the first ten adjusted generations of selection (see text) and selection differentials.

adjusted generations of selection	cumulated mean responses	selection differentials
1	3.1	82.2
2	1.9	79.1
3	4.8	80.3
4	2.9	77.4
5	4.8	79.3
6	8.6	77.4
7	9.2	73.6
8	26.9	73.0
9	16.3	55.3
10	33.5	65.9

Table 41

PSP variation between L-line and LT1-line. The t-test was used to compare mean PSPs and probability values p were determined by a t-table.

generations of selection	P
1	< .9
2	< .3
3	< .05 *
4	< .01 *
5	< .1
6	< .5
8	< .3
9	< .1
10	< .9
11	> .9
12	> .9
13	< .01 *
14	< .9
15	< .5
16	< .9
17	< .2
18	< .5
19	< .3

* indicates a difference significant at p < .05.

Table 42

PSP variation between L-line and LT2-line.T-test and probability values p as in table 41.

generations of selection (of L-line)	P
25	< .2
26	< .9
27	< .9
28	< .5
29	< .5

Table 43

Cumulated mean responses to selection of D1-line calculated over the first ten adjusted generations of selection (as for L-line) and selection differentials.

adjusted generations of selection	cumulated mean responses	selection differentials
1	- 1.5	17.8
2	4.2	19.3
3	5.4	13.6
4	4.0	12.4
5	8.4	13.8
6	7.0	9.4
7	3.9	10.8
8	3.0	13.9
9	8.0	14.8
10	3.9	9.8

Table 44

Cumulated mean responses to selection of D2-line calculated over the first eight generations of selection and selection differentials.

generations of selection	cumulated mean responses	selection differentials
1	1.2	28.6
2	1.3	27.4
3	7.0	27.3
4	13.8	21.6
5	10.0	14.8
6	8.8	18.6
7	12.2	19.8
8	10.0	16.4

Table 45

PSP variation between L-line and D1-line. T-test and probability values p as in table 41.

generations of selection	P
1	> .9
2	< .1
3	< .01 *
4	< .01 *
5	< .01 *
6	< .01 *
8	< .3
9	< .05 *
10	< .01 *
11	< .001 *
12	< .01 *
13	< .001 *
14	< .001 *
15	< .01 *
16	< .001 *
17	< .001 *
18	< .001 *
19	< .02 *
20	< .01 *
21	< .01 *
22	< .01 *
23	< .01 *

* indicates a difference significant at p < .05.

Table 46

Cumulated mean responses to selection of DT1-line calculated over the first ten adjusted generations of selection (as for L- and D1-lines). Corresponding selection differentials and slopes of successive responses.

adjusted generations of selection	cumulated mean responses	selection differentials	slopes of successive responses
1	- 0.3	17.8	- 0.3
2	0.8	18.1	1.1
3	8.8	17.0	8.0
4	7.0	9.0	- 1.8
5	11.1	10.8	4.1
6	13.0	6.7	1.9
7	12.1	4.8	- 0.9
8	10.7	5.7	- 1.4
9	15.8	7.1	5.1
10	16.8	2.0	1.0

Table 47

PSP variation between D1-line and DT1-line. T-test and probability values p as in table 41 (selection was relaxed at generation 7).

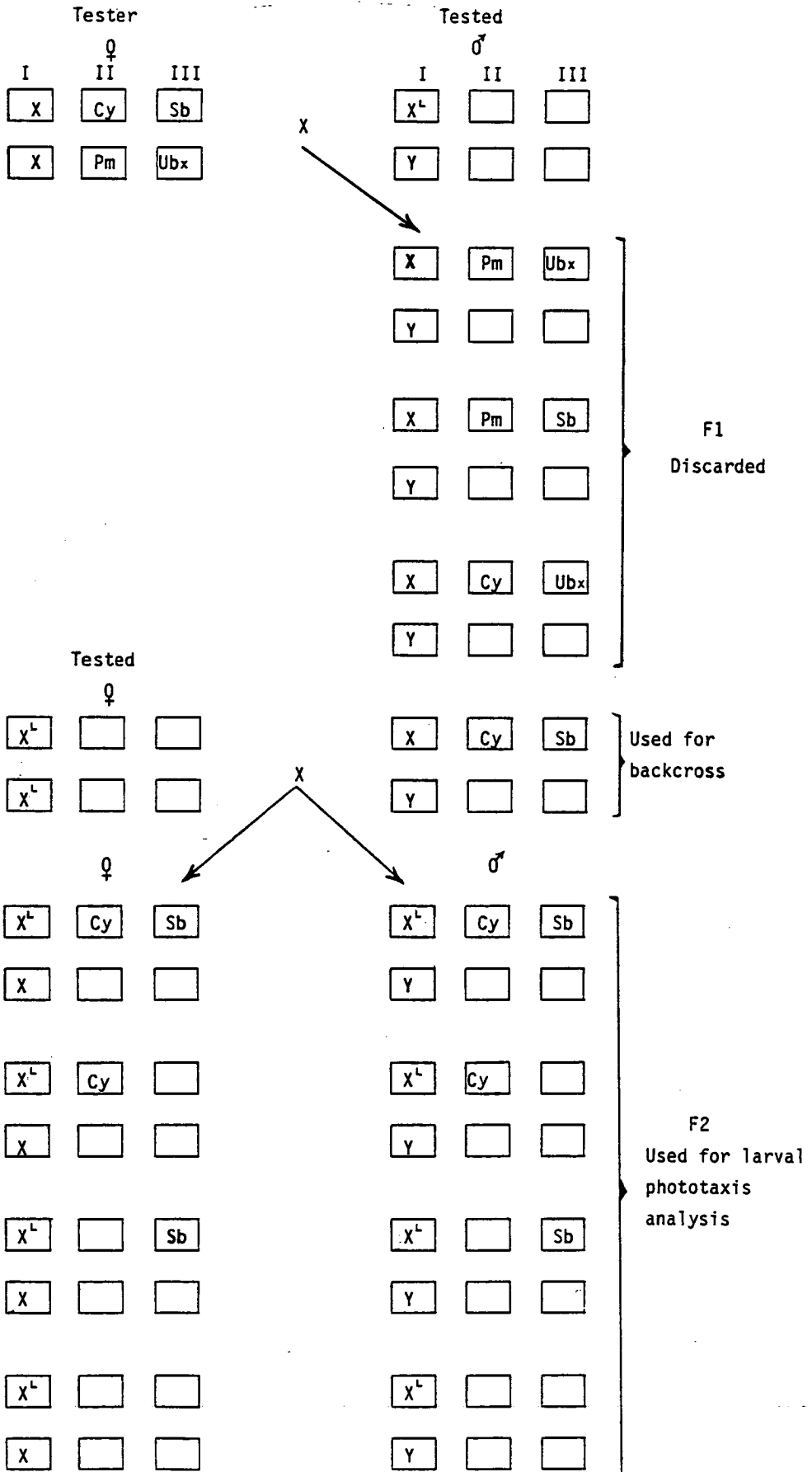| generations of selection	P
1	$< .9$
2	$< .5$
3	$< .2$
4	$< .5$
5	$< .3$
6	$< .3$
8	$\sim .02$ *
9	$< .2$
10	$< .1$
11	$< .01$ *
12	$< .001$ *
13	$< .01$ *
14	$< .001$ *
15	$< .02$ *
16	$< .01$ *
17	$< .001$ *
18	$< .001$ *
19	$< .01$ *
20	$< .001$ *
21	$< .001$ *
22	$< .01$ *
23	$< .01$ *

* indicates a difference significant at $p \leqslant .02$.

Table 48

Cumulated mean responses to selection of DT2-line calculated over the first eight generations of selection. Corresponding selection differentials and slopes of successive responses.

generations of selection	cumulated mean responses	selection differentials	slopes of successive responses
1	3.0	28.6	3.0
2	7.2	25.6	4.2
3	10.2	21.4	3.0
4	16.3	18.4	6.1
5	18.5	12.3	2.2
6	15.0	10.1	- 3.5
7	21.7	13.6	6.7
8	18.9	6.9	- 2.8

Table 49

PSP variation between D2-line and DT2-line. T-test and probability values p as in table 41.

generations of selection	P
1	< .2
2	< .1
3	< .3
4	< .5
5	< .1
6	< .05
7	< .02
8	< .05

Table 50

Intra-sub DT1-line PSP variation. T-test and probability values p. as in table 41.

mean PSPs compared	P
sub $DT1_{20}$* vs sub $DT1_{21}$	< .9
sub $DT1_{20}$ vs sub $DT1_{22}$	< .5
sub $DT1_{21}$ vs sub $DT1_{22}$	< .3

* the number refers to the generation considered.

Table 51

Intra-sub D1-line PSP variation. T-test and probability values p as in table 41.

mean PSPs compared	P
sub $D1_{21}$* vs sub $D1_{22}$	~ .3
sub $D1_{21}$ vs sub $D1_{23}$	< .9
sub $D1_{22}$ vs sub $D1_{23}$	< .5

* the number refers to the generation considered.

Table 52

Mating scheme for investigation of chromosomal difference between D2-
and DT2-lines. See text for the symbols used.

♂♂	♀♀	♂♂	♀♀
D2 (tested)	Tester	DT2 (tested)	Tester

D2 (tested) ♂♂

I	II	III
X^D	D	D
Y	D	D

Tester ♀♀

I	II	III
X	C_y	Sb
X	Pm	Ubx

all Pm and Ubx
phenotypes
discarded

X →

I	II	III
X	C_y	Sb
Y	D	D

I	II	III
X^D	C_y	Sb
X	D	D

all Cy and Sb
phenotypes
discarded

X →

I	II	III
X^D	D	D
Y	D	D

I	II	III
X^D	D	D
X	D	D

I	II	III
X	D	D
Y	D	D

I	II	III
X	D	D
X	D	D

tested for PSP

DT2 (tested) ♂♂

I	II	III
X^{DT}	DT	DT
Y	DT	DT

Tester ♀♀

I	II	III
X	C_y	Sb
X	Pm	Ubx

all Pm and Ubx
phenotypes
discarded

X →

I	II	III
X	C_y	Sb
Y	DT	DT

I	II	III
X^{DT}	C_y	Sb
X	DT	DT

all Cy and Sb
phenotypes
discarded

X →

I	II	III
X^{DT}	DT	DT
Y	DT	DT

I	II	III
X^{DT}	DT	DT
X	DT	DT

I	II	III
X	DT	DT
Y	DT	DT

I	II	III
X	DT	DT
X	DT	DT

tested for PSP

Table 53

Intra -C-line (control-line) PSP variation. T-test and probability values p as in table 41.

mean PSPs compared	P
C_1^* - C_4	< .3
C_1 - C_8	< .9
C_4 - C_8	< .9

* the number refers to the generation considered.

Table 54

Reciprocal hybridizations between L-line and D1-line. PSP of progeny (mean percent of pupae in dark) and standard errors.

	♂	
	L	D1
L	55.1 (±0.8) ★	79.9 (±0.5)
D1	80.5 (±1.0)	87.1 (±1.8)

♀ (left of L/D1 rows)

generation 13

	♂	
	L	D1
L	55.8 (±4.9) ★	77.6 (±2.4)
D1	73.2 (±0.8)	79.7 (±2.2)

♀

generation 19

	♂	
	L	D1
L	57.1 (±3.5) ★	70.8 (-)
D1	60.7 (-)	72.1 (±5.0)

♀

generation 19

	♂	
	L	D1
L	51.3 (±3.3) ★	77.6 (±1.6)
D1	71.4 (±2.3)	83.4 (±2.5)

♀

generation 23

▲
(pupae distribution in the gradient apparatus used for D. simulans)

★ indicates a difference significant at the .05 level.

Table 55

PSP variations between progeny of the reciprocal hybridizations between L-line and D1-line. T-test and probability values p as in table 41.

variation between	P (generation 13)	P (generation 19)	P (generation 23)
♀ L x ♂ D1 progeny and ♀ D1 x ♂ L progeny	< .9	< .2	< .1
variation between ♀ L x ♂ D1 progeny and L	< .001	< .02	< .01
variation between ♀ L x ♂ D1 progeny and D1	< .02	< .9	< .2
variation between ♀ D1 x ♂ L progeny and L	< .001	< .02	< .01
variation between ♀ D1 x ♂ L progeny and D1	< .05	~ .05	< .05

Table 56

Sex ratios of light and dark preferring progeny issued from L-line x D1-line reciprocal hybridizations. Comparison of sex ratios between extreme light and extreme dark preferring larvae were made by using a 2 x 2 contingency table.

generation of selection	crossing pattern	% of progeny in extreme light		% of progeny in extreme dark		number of observations	dead pupae
		♂	♀	♂	♀		
19	♀L x ♂ D1	67.8	32.2	44.2	55.8	155**	1
19	♀ D1 x ♂ L	49.1	50.9	46.9	53.1	120	3
23	♀ L x ♂ D1	59.0	41.0	43.4	56.6	165*	5
23	♀ D1 x ♂ L	43.3	56.7	-	-	61	1

* indicates a difference at p ∼ .05.
** indicates a difference at p < .01.

Table 57

Reciprocal hybridizations between D1-line and DT1-line. PSPs of progeny (mean percent of pupae in dark) and standard errors.

generation 14

generation 21

★ indicates a difference significant at the .05 level.

Table 58

PSP variations between progeny of the reciprocal hybridizations between D1-line and DT1-line. T-test and probability values p as in table 41.

variation between	p(generation 14)	p(generation 21)
♀ D1 x ♂ DT1 progeny		
and	< .01	< .1
♀ DT1 x ♂ D1 progeny		
variation between		
♀ D1 x ♂ DT1 progeny		
and	< .001	< .02
D1		
variation between		
♀ D1 x ♂ DT1 progeny		
and	< .5	< .01
DT1		
variation between		
♀ DT1 x ♂ D1 progeny		
and	< .9	< .3
D1		
variation between		
♀ DT1 x ♂ D1 progeny		
and	< .01	< .01
DT1		

Table 59

Reciprocal hybridizations between LT2-line and D1-line. PSP of progeny (mean percent of pupae in dark) and standard errors.

generation 29

★ indicates a difference significant at the .05 level.

Table 60

PSP variations between progeny of the reciprocal hybridizations between LT2-line and D1-line at generation 29. T-test and probability values p as in table 41.

variation between   p(generation 29)
♀ LT2 x ♂ D1 progeny
       and   < .1 (close to .05)
♀ D1 x ♂ LT2 progeny

variation between
♀ LT2 x ♂ D1 progeny
       and   < .05
      LT2

variation between
♀ LT2 x ♂ D1 progeny
       and   < .02
      D1

variation between
♀ D1 x ♂ LT2 progeny
       and   < .02
      LT2

variation between
♀ D1 x ♂ LT2 progeny
       and   < .5
      D1

Table 61

Sex ratios of light and dark preferring progeny issued from LT2-line x D1-line reciprocal hybridizations at generation 29 of selection.

crossing pattern	% of progeny in extreme light		% of progeny in extreme dark		number of observations	dead pupae
	♂	♀	♂	♀		
♀ LT2 x ♂ D1	55.0	45.0	42.9	57.1	254	4
♀ D1 x ♂ LT2	45.8	54.2	50.3	49.7	258	8

Table 62

Comparison of the percentages of light and dark preferring males (of table 61) with an expected 50 %. Probability values p (that the observed percentages differ from 50 %) were determined by a standard normal table.

generation of selection	crossing pattern	P in extreme light	P in extreme dark
29	♀ LT2 x ♂ D1	~.32	~.06
29	♀ D1 x ♂ LT2	~.52	~.93

Table 63

Sex ratios of light and dark preferring progeny issued from tester females x L-line tested males, crossed at generation 30 of selection. Probability values p (that the observed percentages differ from 50 %) were determined by a standard normal table.

% of progeny in extreme light		% of progeny in extreme dark		number of observations	dead pupae	P (extr. light)	P (extr. dark)
♂	♀	♂	♀				
66.7	33.3	44.8	55.2	326	13	~ .01	~ .09

Table 64

Genotype frequencies in light and dark of progeny issued from L-line tested females x tester males, crossed at generation 31 of selection. Probability values p (that the observed percentages differ from 25 %) were determined by a standard normal table.

	genotype	total frequency (%)	frequency in light (%)	P (in light)	frequency in dark (%)	P (in dark)
♂	Cy Sb	24.5	14.9	< .01	34.4	< .01
	Cy -	23.0	22.0	~ .12	23.9	~ .57
	- Sb	25.4	23.2	~ .35	27.6	~ .18
	- -	27.1	39.9	< .01	14.1	< .01
♀	Cy Sb	26.1	19.2	< .01	31.5	< .01
	Cy -	24.7	32.7	< .01	18.5	< .01
	- Sb	25.3	18.0	< .01	31.0	< .01
	- -	23.9	30.1	~ .01	19.0	< .01

Table 65

Comparisons of the ratios of light and dark preferring larvae between the different phenotypes of table 64. 2 x 2 contingency tables were used and probalility values p were determined by a χ^2 table. — represents a chromosome from the L-line.

II	III		II	III	χ^2	P	χ^2	P
				♂♂			♀♀	
Cy	—	vs	Cy	Sb	5.211	< .05	12.075	< .001
Cy	—	vs	—	—	11.685	< .001	0.125	< .9
—	Sb	vs	Cy	Sb	4.207	< .05	0.028	< .9
—	Sb	vs	—	—	14.324	< .001	10.439	< .01

Table 6.6

Sex ratios of light and dark preferring progeny issued from L-line tested females x tester males, crossed at generation 31 of selection. Probability values p (that the observed percentages differ from 50 %) were determined by a standard normal table.

% of progeny in extreme light		% of progeny in extreme dark		number of observations	dead pupae	P (extr. light)	P (extr. dark)
♂	♀	♂	♀				
51.9	48.1	44.9	55.1	709	22	~ .49	~ .05

Table 67

Mean egg-larval mortality of L-, LT1-, LT2-, D1-, D2-, DT1- and DT2-lines calculated over the whole selection experiment.

selected lines	number of eggs examined	mean egg -larval mortality (%)
L-line	7572	6.5
LT1-line	4943	8.5
LT2-line	1396	6.9
D1-line	6219	5.8
D2-line	2173	5.5
DT1-line	6047	8.4
DT2-line	2256	6.0

Table 68.

Mean mortality at the pupal stage of L-, LT1-, LT2-, D1-, D2-, DT1- and DT2-lines calculated over the whole selection experiment.

selected lines	number of pupae examined	mean mortality (%)
L-line	1400	3.7
LT1-line	900	4.8
LT2-line	250	2.8
D1-line	1100	2.4
D2-line	526	2.4
DT1-line	1100	3.8
DT2-line	579	3.1

Table 69

Percentages of males found among the selected flies of L-, LT1-,
D1-, D2-, DT1- and DT2-lines. Probability values p (that these
percentages differ from 50 %) were determined by a standard normal
table.

selected lines	number of flies examined	% males	P
L-line	228	45.6	$\sim$ .18
LT1-line	226	44.3	$\sim$ .08
D1-line	243	47.3	$\sim$ .40
D2-line	514	51.8	$\sim$ .43
DT1-line	230	37.8	$<$ .001
DT2-line	563	44.8	$\sim$ .01

Table 70

PSPs (mean percent in dark) of IC-, IPL- and IPD-lines and of progeny from the reciprocal hybridizations between IPL- and IPD-lines. The t-test was used to compare mean PSPs and probability values were determined by a t-table.

generation number	IC- line	IPL- line	IPD- line	IPL vs IPD P	♀ IPL x ♂ IPD (I)	♀ IPD x ♂ IPL (II)	I vs II P
4	74.6	78.9	74.4	< .2	-	-	-
5	-	-	-	-	85.8	80.7	< .2
7	-	77.8	73.1	< .1	-	-	-
8	-	-	-	-	84.5	82.7	< .5

Table 71

Mean PSPs (mean percent in dark) of ID- and IDT-lines. The t-test was used to compare mean PSPs and probability values p were determined by a t-table.

generations of selection	ID-line	IDT-line	P
1	72.9	-	-
2	75.5	-	-
3	73.7	72.7	< .9
4	74.3	74.5	> .9
5	71.9	75.1	< .3
6	73.4	74.4	< .9
7	71.6	74.8	< .3
8	75.4	73.1	< .5

Table 72

OSPs of L-, Dl-, DT1-lines and of progeny issued from the reciprocal hybridizations between L-line and Dl-line.
Arcsin √proportion of eggs laid in dark and 95 % confidence limits (C. L.).

generation of selection	line tested	arcsin √% eggs laid in dark	95 % C.L.
18	L-line	50.3	± 7.2
18	Dl-line	67.4	± 7.8
28	L-line *	72.4	± 6.9
28	Dl-line *	80.0	± 4.3
28	DT1-line *	76.7	± 4.2
19	♀ L x ♂ D1 progeny	59.7	± 7.4
19	♀ D1 x ♂ L progeny	63.4	± 5.7

* Tests were conducted under different conditions of light (see text for explanation).

Table 73

OSP of progeny issued from the reciprocal hybridizations between L-line and D1-line. Arcsin $\sqrt{\text{proportion of eggs laid in dark}}$ and 95 % confidence limits.

generations 18 and 19

★ indicates a difference significant at the .05 level.

Table 74

OSPs of both "light" and "dark" preferring females, selected from the base population.

Arcsin $\sqrt{\text{proportion of eggs laid in dark}}$ and 95 % confidence limits (C. L.).

PSPs of the progeny of these two groups and standard errors (S. E.). The t-test was used to compare mean PSPs and probability value p was given by a t-table.

Group of females	arcsin $\sqrt{\text{\% eggs}}$ laid in dark	95 % C.L.	Progeny PSPs (% in dark)	S.E.	P
"light" preferring females	40.0	±11.8	74.4	±1.1	<.01
"dark" preferring females	77.2	± 6.8	85.0	±1.7	

Table 75

Summary of the changes in PSP through generations in the main lines studied. Estimates of the "changes" are measured as responses (R) to selection over the generations listed.

lines	change in PSP(R)	generations over which R was calculated
L-line	2.816	1-10
LT1-line	2.694	1-10
D1-line	0.490	1-10
D2-line*	1.517	1-8
DT1-line	1.734	1-10
DT2-line*	2.593	1-8
C-line	no change	1-8
PL-line*	no change	1-7
PD-line*	slightly more photopositive	1-7
IC-line*	no change	1-4
IPD-line*	no change	1-8
IPL-line*	no change	1-8
ID-line*	no change	1-8
IDT-line*	no change	1-6

*a different experimental design was used to assess PSP (see text).

CHAPTER 4 : CHEMOPREFERENCES IN D. MELANOGASTER

4.1 - Introduction

Alcolhol is known to affect relative species numbers of Drosophila in the wild, especially in sites such as fermentation areas of wineries (Parsons, 1979). It was mentioned under 1.9 that Drosophila exploits resources released by fermentation and decay and therefore ethanol resource utilization among Drosophila species attracted to fermented-fruit baits has been studied extensively. Mc-Kenzie and Parsons (1974) observed that D. simulans apparently does not utilize alcohol as a resource, as opposed to D. melanogaster. The latter species then shows a markedly higher tolerance to environmental alcohol than the former (David et al., 1974), and it was further stressed in chapter 1 that this discrepancy is paralleled by larval preferences (Parsons, 1977). As McKenzie and McKechnie (1979) found that high ethanol and acetic acid concentrations in grape residues are correlated, D. mela-nogaster initially appeared to be a favourable species to use for the investigation of larval preferences for both ethanol and acetic acid.

Lines were then selected for positive and negative preferences for both the above products. From each of these four lines, traumatic lines were established in

accordance with the general scheme outlined in figure 1.
For the practical reasons mentioned under 1.6, early pre-
ferences for chemicals occurring in the culture medium
could not be measured soon after pupation as with photo-
preferences. Early third and occasionally late second in-
star larva preferences were assessed instead. This lack
of precision was due to the fact that the introduction of
a vertical separation (see below) could not be made when
all the larvae were of exactly the same age. Slight differ-
ences in mean environmental temperature or in the amount
of living yeast at the surface of the medium inevitably
modified the developmental rate at some generations so
that it was not possible to ascertain the exact age of the
larvae at the time the preferences were recorded.

Since yeast and bacteria (on which the young lar-
vae were feeding) did not grow with the same speed on me-
dia with or without the chemicals used this was bound to
make the comparison of larval preferences between lines
difficult. It can thus be argued that eggs issued from
adults which were exposed to ethanol vapours for instance
should be expected to carry less microorganisms than eggs
issued from adults which were not exposed to the chemical.

4.2 - Alcohol preferences (first trial)

4.2.1 - Methods and strain used

One hundred females coming from the base population of D. melanogaster used for the selection of larval photopreference (see under 3.2.2) were pooled with one hundred females derived from a Prevosti strain, which had been initiated from a large collection made in the Canary Islands by Professor A. Prevosti. The flies were kept together for four generations in a population cage at 25°C $\pm$ 1°C to form the base population.

4.2.2 - Method of egg collection

Experiments were initiated by eighty fertilized females issuing from the base population, being allowed to lay eggs in egg collection pots (∅ 30 mm) containing 2 mm of fresh standard medium without living yeast. Narrow strips of medium about 20 to 30 mm long were sliced out, washed in distilled water and deposited in the apparatus for measuring larval preferences.

4.2.3 - Experimental design used for the selection of alcohol preferences

Transparent glass staining dishes of the same size as those used for the investigation of phototaxis

(see figure 12) were used throughout. One half of the
dishes contained 20 mm of standard medium supplemented
with absolute ethanol 6% by volume. The ethanol was added
to the cooling down phase of preparation of the medium,
between 50°C and 55°C. The lids of the dishes were made of
transparent plexiglas with two foam stoppers as shown in
figure 12. One hundred eggs were deposited along the mid-
line of the dishes before these were left in total dark-
ness. Sixty-five hours after laying the dishes were opened
in reduced light and a vertical separation (0.3 mm thick)
was introduced into the medium in order to prevent further
larval migration from one half to the other. The larvae
were then allowed to pupate under permanent white light,
to prevent them from crawling on the walls or the lids of
the dishes. The majority of the larvae thus pupated at the
surface of the medium although a few pupae were repeated-
ly found on the low part of the walls. The selected pupae
were then mostly removed from the surface of the medium.
To further ensure that a high proportion of larvae pupated
at the surface of the medium, favourable humidity condit-
tions were provided by opening the lids of the dishes for
several periods between hour sixty-five and pupation time.
It is indeed well established (e.g. Sokal et al., 1960 ;
Sameoto and Miller, 1967) that an increased water content
of the medium in vials normally used to rear small numbers
of flies causes an increase in the proportion of pupation
on the walls of the vials.

4.2.4 - <u>Selection and environmental conditions of the four</u>
<u>selected lines</u>

As in the experiments on phototaxis three dishes
were always run simultaneously in each line and the pro-
cedure of selection was identical to that described for
photopreferences in <u>D</u>. <u>melanogaster</u>. One hundred and
twenty to one hundred and twenty-five hours after laying,
fifty to sixty pupae were removed from the medium with a
paintbrush, put in pairs on the surface of 8cc of stand-
ard medium in vials in which either ethanol or distilled
water had been poured in a central cylindric hole (∅ 5mm).
The amount of vapour breathed by the pupae was not meas-
ured but the 0.6 ml of solution poured in each vial eva-
porated in about four days. The pupae were all kept under
a 12 hour light/12 hour dark cycle and exposed to the
following environmental conditions :

- Line selected for alcohol preference (AL-
line) : 0.6 ml 60 % ethanol was poured in the central hole.

- Line selected for alcohol preference ex-
periencing an environmental traumatic treatment (ALT-line):
0.6 ml distilled water was poured in the central hole.

- Line selected for negative alcohol prefer-
ence (NAL-line) : 0.6 ml distilled water was poured in the
central hole.

- Line selected for negative alcohol prefer-

ence experiencing an environmental traumatic treatment
(NALT-line) : 0.6 ml 60% ethanol was poured in the central
hole.

At eclosion the flies were sexed and twenty
couples (to be used as parents of the next generation)
were put as soon as possible in twenty vials containing
standard medium provided with a drop of living yeast sus-
pension. Prepupal mortality as well as mortality at the
pupal stage were estimated at each generation.

4.2.5 - Results

Figure 15 shows that AL-line responded to se-
lection: for the six generations considered the response
to selection was R = 2.150 and the coefficient of cor-
relation between R and progress of selection is significant
at the .01 level when tested against ρ = 0. The realized
heritability was h^2 = 0.056 which is significant at the
.05 level when tested against β = 0. ALT-line, however,
apparently showed no such change (R =-0.539, correlation
coefficient not significant) but the sample variances
were so large in both lines that the preferences of the
two lines were actually not significantly different from
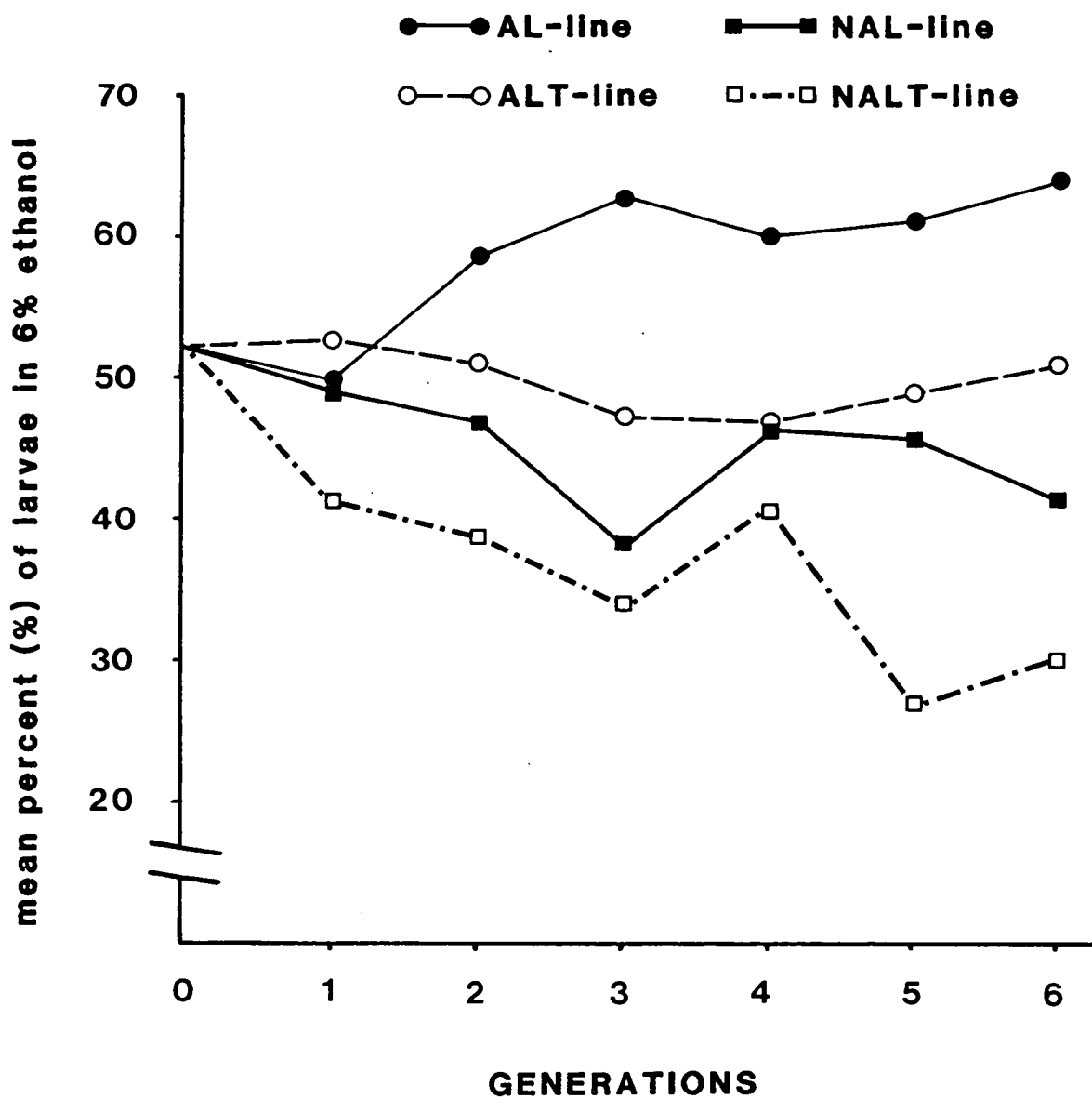each other when compared between simultaneous generations
(table 76, p. 228).

Figure 15. Mean percent of larvae in 6% ethanol compartment plotted against generations of selection.

A rather reversed situation was observed among NAL-line and NALT-line (figure 15). Here the former did not respond to selection (R = 1.461) ; h^2 = 0.024, both are not significant at the .05 level),whereas the latter showed a stronger response (R = 3.343, h^2 = 0.067, significant at the .01 and .1 levels respectively). Nevertheless, the sample variances were so large again that it cannot be concluded that the larval preferences of these two lines were actually different (table 76).

The abnormally high mortality (as egg, larva and pupa) observed in all four lines (table 77) made the results of this first experiment particularly unreliable. Part of the larval mortality was probably due to the experimental procedure, since the introduction of the vertical separation into the medium was bound to hurt or kill a few larvae. None of the sex-ratios reported in table 77 indicated a significant departure from an expected 1 : 1 ratio.

The differences in preference observed between AL-, ALT- and NAL-lines can perhaps be accounted for by mere differences in the microflora available for the larvae, as mentioned earlier. Thus the treatment by alcohol vapours was thought to cause a substantial decline in the amount of the "microbial luggage" naturally carried by the pupae. As yeast and bacteria could grow faster on medium without alcohol, more larvae might then have been

attracted by the halves of the dishes free of alcohol in lines where the microorganisms were relatively more abundant, like in NAL- and ALT-lines, experiencing distilled water vapours instead of alcohol.

Interestingly, the salient exception to this way of reasoning would be NALT-line, which showed an increasing preference for medium deprived of alcohol, even though there was presumably less microflora available as the trauma was further applied, compared with what NAL-line larvae were simultaneously experiencing. Nonetheless, in order to counterbalance any such effect, a procedure called "microflora compensation" was devised in a second trial of selection for alcohol preferences. In addition, another stock of flies was used for this second experiment, since the high mortality reported above was suspected to have perhaps been caused by hybrid dysgenesis which could have occurred in the base population as a result of an interaction between the two strains used.

4.3 - Alcohol preferences (second trial)

4.3.1 - Methods and strain used

The stock of recently caught D. melanogaster which was given to me by Dr. S. Newbury (see under 3.2.2) was kept for six months in a population cage at $25°C \pm 1°C$

and formed the base population used for the second experiment on alcohol preferences. Experiments were initiated by eighty fertilized females issuing from this population and both egg collection and egg deposition were conducted as in the previous experiment.

4.3.2 - Microflora compensation

At generations 3, 5, 7, 8 and 9 the selected females of AL-line and NALT-line (i.e. of the lines poor in microbial luggage because of the toxicity of alcohol) were allowed to feed upon plenty of microflora removed from the six halves without alcohol of the dishes of NAL-line and ALT-line (i.e. of the lines more rich in microbial luggage). These yeast and bacteria were removed from the dishes soon after removal of pupae and they were grown on standard medium in one-third pint milk bottles. The females destined to benefit from microflora compensation were introduced in these bottles for twenty-four hours just before egg collection.

4.3.3 - Selection and environmental conditions of the four selected lines

The procedure of selection was identical to the previous one except that two points relative to the environmental conditions of both larvae and pupae were mo-

dified as follows :

- The halves of the dishes containing alcohol were supplemented with absolute ethanol 5% (instead of 6%) by volume in order to lessen the inhibitory effect of alcohol on microbial growth.

- The central hole of the vials containing the selected pupae of AL-line and NALT-line contained 0.6 ml of 50% ethanol (instead of 60%) in order to lessen the effect of alcohol vapours on microorganisms carried by the pupae.

4.3.4 - Results

Figure 16 shows that this second experiment equally indicated that attempts to detect differences in preference between rewarded and traumatic lines for either positive or negative preferences seemed to fail. AL-line responded more weakly to selection than in the previous experiment. For the ten generations considered the response was $R = 0.673$ and the correlation coefficient between R and progress of selection is just significant at the .05 level when tested against $\rho = 0$. The realised heritability was $h^2 = 0.009$ which is not significant when tested against $\beta = 0$ (p < .5). As in the previous experiment ALT-line larvae tended to show less strong preference for medium containing alcohol than AL-line. Again
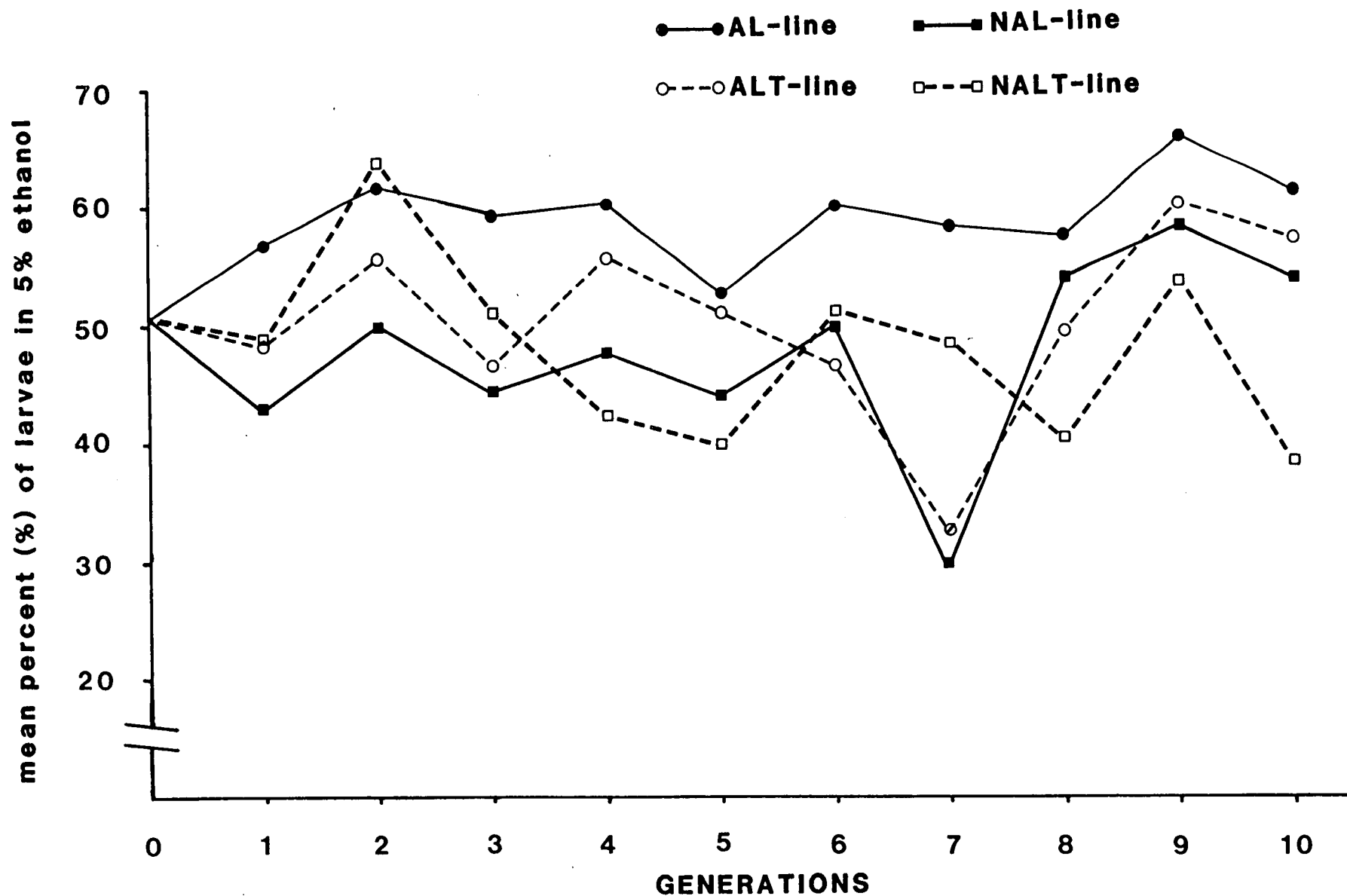
Figure 16. Mean percent of larvae in 5 % ethanol compartment plotted against generations of selection.

the sample variances were so large that at most generations the preferences of the two lines were not significantly different from each other.

Similarly to what was observed in the previous experiment NAL-line showed no response to selection (R = 0.555 ; h^2 = 0.0194, both are not significant). Regrettably the only possibly interesting result relative to NALT-line (as observed in the previous experiment at least) could not be properly replicated as this line showed a very high mortality at the pupal stage at the outset of the experiment (table 78). This was so high that the traumatic treatment was not applied at generation 1. Figure 16 shows that the mean alcohol preferences of NALT-line remained relatively close to those of NAL-line throughout. The egg-larval mortality was about 10% in all lines, which is approximately half what was observed in the first trial.

The absence of a good response either way in this second experiment is difficult to understand. Ethanol is expected to be used by D. melanogaster larvae as a food resource compound, whose likelihood of use as a nutrient might depend on the presence of a single mutation (fast or slow allele at the dehydrogenase locus), although the evidence here is far from clear, as judged from results of experiments on alcohol tolerance (e.g. McKenzie and Mc-Kechnie, 1978).

With respect to this the second chemical I used
(namely acetic acid) had perhaps more promise to show
an effect analogous with what was observed with light, as
this chemical is thought to act as a recognition compound
as well as a food resource (Parsons and Spence, 1981).
This might then indicate that preference behaviour for
this product is more likely to be polygenically controll-
ed than preference behaviour for ethanol. Besides, the low
volatility of acetic acid (boiling point at 118°C) allow-
ed a better control of the concentrations used between
different experimental trials.

4.4 - Acetic acid preferences

4.4.1 - Methods and strain used

Eighty fertilized females of $\underline{D}$. $\underline{melanogaster}$
coming from the base population used in the previous ex-
periment (see under 4.3.1) were used in a very similar
selection experiment for acetic acid larval preferences,
also performed at 25°C $\pm$ 1°C. Both egg collection and egg
deposition were conducted as in the selection experiments
for alcohol preferences.

4.4.2 - Microflora compensation

At generations 2, 4, 6, 7 and 8 the same proced-

ure aimed at counterbalancing the loss of microorganisms (this time due to the toxicity of acetic acid) was used as previously described by supplying the lines affected by the acid vapours with microflora coming from the un-affected lines.

4.4.3 - Selection and environmental conditions of the four selected lines

The procedure of selection was identical to that used in the two previous selection experiments for alcohol preferences.

The halves of the dishes containing the acid were supplemented with 2% glacial acetic acid by volume, whereas the other halves were supplemented with the equivalent volume of distilled water. One hundred and twenty to one hundred and twenty-five hours after laying fifty to sixty pupae (mostly pupated at the surface of the medium) were removed with a paintbrush and put in pairs in vials. Pupae and adults of all lines were exposed to a 12 hour light/12 hour dark cycle and treated simultaneously.

From the stage of newly formed pupae up till four or five days old adults the animals were thus exposed in vials to the following environmental conditions :

- Line selected for acetic acid preference (AC-line) : 0.6 ml 2% glacial acetic acid was poured in the central hole of the vials.

- Line selected for acetic acid experiencing an environmental traumatic treatment (ACT-line) : 0.6 ml distilled water was poured in the central hole.

- Line selected for negative acetic acid preference (NAC-line) : 0.6 ml distilled water was poured in the central hole.

- Line selected for negative acetic acid preference experiencing an environmental traumatic treatment (NACT-line) : 0.6 ml 2% glacial acetic acid was poured in the central hole.

At eclosion the flies were sexed and twenty couples were put as soon as possible in twenty vials all containing standard medium provided with a drop of living yeast suspension. Prepupal mortality as well as mortality at the pupal stage were estimated at each generation.

4.4.4 - <u>Results</u>

4.4.4.1 - <u>Responses to selection of AC-, ACT-, NAC- and NACT-lines</u>
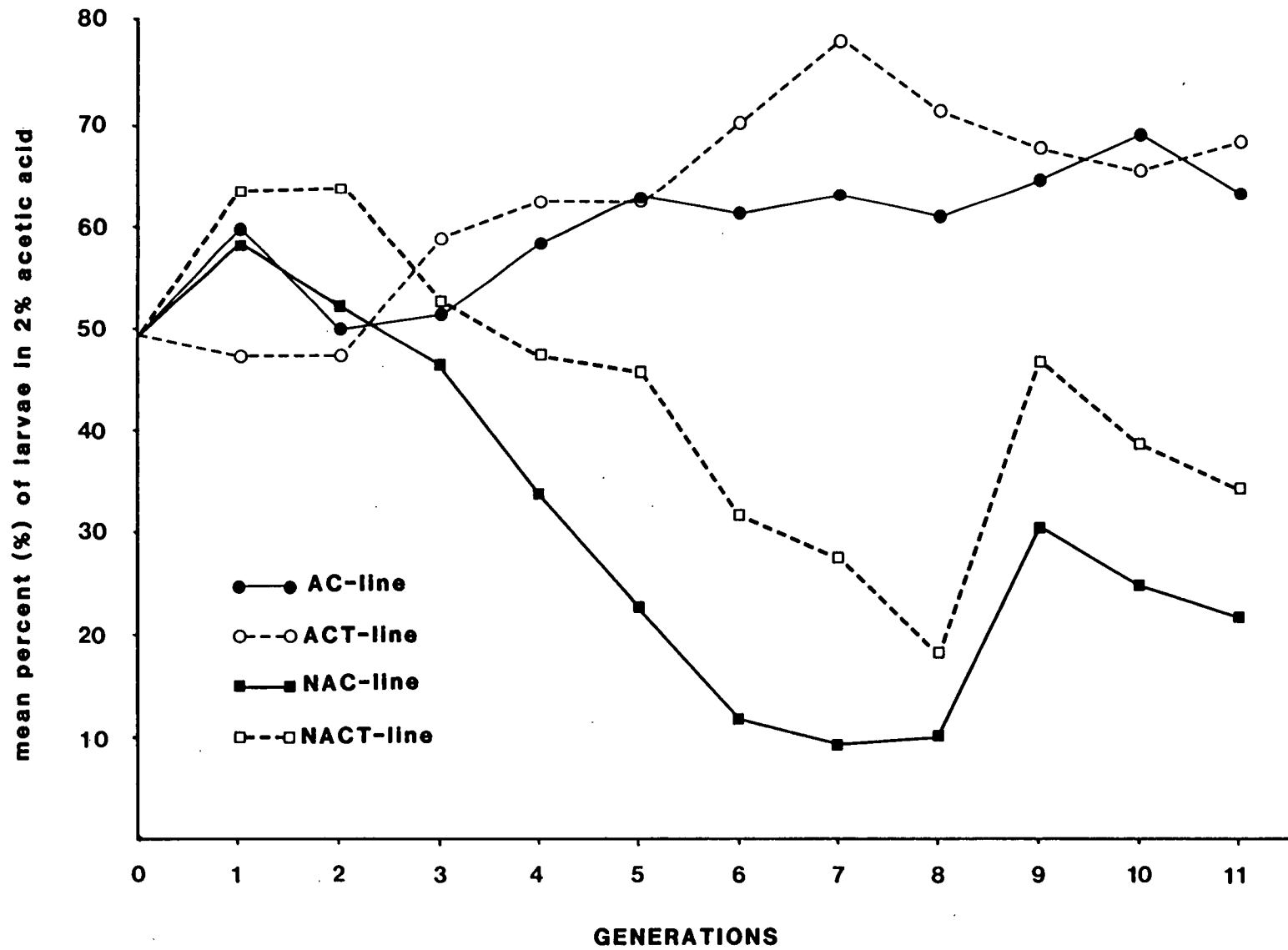
Figure 17 shows that AC-line responded relative-

Figure 17. Mean percent of larvae in 2% acetic acid compartment plotted against generations of selection.

ly weakly to selection. For the eleven generations cons-
idered the average response was R = 1.363 and the co-
rrelation coefficient between R and progress of selection
is significant at the .01 level when tested against $\rho = 0$.
The realized heritability was h^2 = 0.031 which is signif-
icant at the .01 level when tested against $\beta = 0$.

Larval preferences of ACT-line remained close
to those of AC-line (Figure 17) and at most generations
the standard errors on the respective mean preferences
(not reported in the figure) greatly overlap.

Interestingly NAC-line responded very strongly
to selection, assuming that the sudden change recorded
between generations 9 and 11 can at least partly be
attributed to a temperature effect. During these gener-
ations the dishes of all four lines had to be run in a
different incubator room where the temperature was 23°C
instead of 25°C. This apparently affected the larval pre-
ferences of NAC-line and NACT-line only. It is thus
possible that the larvae of these lines were less strong-
ly attracted by media without acid than in previous gener-
ations because they had relatively less microbes avail-
able,owing to a general slowing down of the microbial
growth rate at 23°C compared with 25°C.

Nevertheless,the response to selection of NAC-
line up to generation 8 was R = 6.840, which is signific-

ant at the .001 level when tested against $\rho = 0$. The realized heritability of this line calculated over the same generations was $h^2 = 0.227$, which is significant at the .001 level too when tested against $\beta = 0$ and is by far the highest value obtained in my selection for chemical preferences.

Table 79 shows that NACT-line larvae tended to have more acetic acid preference than NAC-line larvae after five generations of selection accompanied by the traumatic treatment. Between generations 5 and 11 of selection, five cases (of comparison of dish distributions) out of seven were significantly different at the .05 level using the t-test. The reasoning outlined about a possible difference in food availability between NAL-line and NALT-line (under 4.2.5) would lead here to a difference between ACT- and NACT-line in the direction shown by the results. However the microflora compensation (carried out at least one generation on two) makes it unlikely that consistent differences in microbial populations could have been maintained between these two lines. The possibility that the observed difference was merely due to genetic drift still cannot be ruled out as there were no replicates of the above lines.

4.4.4.2 - <u>AC-line X NAC-line reciprocal hybridizations</u>

At generations 8 and 10 of selection reciprocal

hybridizations were carried out between AC-line and NAC-
line, using the same numbers of parents as in the crosses
of lines selected for light preferences. The summarized re-
sults are presented in table 80, and table 81 shows that
the larval preferences of progeny issued from the reciproc-
al hybridizations were significantly different at the .01
level using the t-test. The results thus indicated that
preferences for medium with acetic acid were transmitted
maternally, as the preference of progeny from AC-line
females X NAC-line males were not different from those of
AC-line, as opposed to the preference of the progeny from
the reciprocal cross. The emerging picture is thus com-
patible with an X-linked dominant genetic basis controll-
ing acetic preference, unless a strong maternal effect can
account for the results.

Table 82 shows that the percentages of males and
females issued from the above reciprocal hybridizations
found in both types of medium did not depart from a 1 : 1
ratio. This is an unexpected result on the basis of the
X-linkage just postulated as this would have implied that
most male larvae issued from NAC-line female X AC-line
male cross should have preferred the medium without acetic
acid, while most females should have preferred the medium
with acetic acid. This was apparently not the case ($p \sim .31$
and $p \sim .92$ respectively) and more attention will be paid
to this observation in the discussion of this chapter (4.
4.5).

4.4.4.3 - <u>Larval mortality and mortality at the pupal</u>
<u>stage of the selected lines</u>

Table 83 shows that the mean egg-larval mortality was low,although the greatest inter-line variation observed was precisely that between NAC-line and NACT-line which apparently yielded interesting results. The same applies to the mortality recorded at the pupal stage but it seems safe to assume that in both cases these small differences are unlikely to have caused the divergence in preferences which took place in less than five generations.

4.4.5 - <u>Discussion</u>

The results relative to larval preferences for chemicals turned out less informative than those obtained for larval photopreferences. Several disadvantages of the former experimental designs may partly account for this difference. For example,it must be emphasized that when larvae were selected for preferences for chemicals, the exact cues to which the animals responded at the time the preferences were recorded were much less clear than in the selection experiments for photopreferences. There were perhaps important variations of the concentration of the chemical used through time as well as possible formation of secondary products and this was not investigated. More-

over, the microflora compensation procedure may not have been entirely effective. The obvious way to overcome this difficulty would have been to work on sterile culture media (e.g. Gordon and Sang, 1942), but this would inevitably have slowed down the development rate and survivorship of the larvae and would not have allowed a proper control of the timing of development of the larvae as was required. Nevertheless, the above considerations cannot provide a satisfactory explanation for the absence of strong responses to selection, as was observed in the second selection experiment of larval preferences for alcohol.

Perhaps the most interesting result obtained in these experiments on chemotaxis relates to the pattern of transmission of preference for media containing acetic acid or to the way maternal factors were maybe involved in it. The observed asymmetry of response to selection for positive or negative larval preferences for acetic acid suggests that natural selection may favour preference for substrates containing acetic acid. This is compatible with the view that this compound, even at very low concentrations, can act as an indicator of favourable feeding sites. The control of second or third instar larva preferences for acetic acid could then be expected to involve a good proportion of dominant genes and my results from the reciprocal hybridizations between AC- and NAC-lines may support just such genetic architecture.

Still the absence of biased sex ratio among
hybrid progeny in both types of medium needs an explan-
ation. According to the reasoning outlined in chapters 2
and 3 about a hypothetical involvement of cytoplasmic
factors which could modulate the expression of the above
X-linked dominant genes, the following possibility might
be envisaged, although it was not substantiated by
empirical evidence. Let us suppose that NACT-line larvae
tended to show more preference for media containing acetic
acid relatively to NAC-line because environmental acetic
acid vapours somehow favoured a cytoplasmic factor normal-
ly enhancing the action of X-linked genes (rare in NACT-
line) controlling positive acetic acid preference. It
could then be argued that the absence of such environment-
al vapours might have lessened the action of the postul-
ated factor in a similar way to which darkness was post-
ulated to affect PSP compared with light for photoprefer-
ences. A consequence of this would be that the action of
such cytoplasmic factor would also have been reduced among
progeny from NAC-line female X AC-line male cross. This
could then partly account for the observed absence of
biased sex ratio in media without acetic acid reported
under 4.4.4.2.

TABLE 76

Variation of acetic acid larval preferences between AL- and ALT-lines and between NAL- and NALT-lines. The t-test was used on mean larval preferences and probability values p were determined by a t-table.

generations of selection	P AL vs ALT	P NAL vs NALT
1	< .9	< .3
2	< .3	< .1
3	< .01 *	< .9
4	< .02 *	< .5
5	< .2	< .02 *
6	< .1	< .1

* indicates a difference significant at p < .02.

TABLE 7·7

Egg-larval mortality and mortality at the pupal stage of AL-, ALT-, NAL-and NALT-lines in the first selection experiment for alcohol pre-ferences and sex ratios (♂/♀) estimated from the flies emerging from the selected pupae.

selected lines	egg-larval mortality (%)	mortality at the pupal stage (%)	sex ratio
AL-line	20.4	9.0	1.04
ALT-line	19.4	10.7	0.84
NAL-line	19.1	10.3	0.81
NALT-line	20.0	10.7	0.98

Table 78

Mortality at the pupal stage of AL-, ALT-, NAL- and NALT- lines in the second selection experiment for alcohol preferences (always estimated from fifty selected pupae).

generations of selection	AL-line mortality (%)	ALT-line mortality (%)	NAL-line mortality (%)	NALT- line mortality(%)
0	8	10	6	22
1	4	4	2	32
2	0	4	6	6
3	4	4	2	6
4	4	6	2	2
5	16	2	0	14
6	6	2	2	8
7	2	8	2	4
8	4	0	2	4
9	0	2	4	2
10	4	2	0	2
means (%)	4.7	4.0	2.5	9.3

Table 79

Variation of acetic acid larval preferences between NAC-line and NACT-line. The t-test was used on mean larval preferences and probability values p were determined by a t-table.

generations of selection	P
4	< .2
5	< .05 *
6	< .05 *
7	< .05 *
8	< .1
9	< .05 *
10	< .1
11	< .05 *

* indicates a difference significant at p < .05.

TABLE 80

Reciprocal hybridizations between AC-line and NAC-line. Percentages of progeny larvae found in the halves of the dishes containing 2 % acetic acid and standard errors.

	♂ AC	♂ NAC
♀ AC	64.6 (1.4)	59.9 (1.7)
♀ NAC	43.0 (1.7)	30.6 (3.0)

generation 9

	♂ AC	♂ NAC
♀ AC	63.2 (4.6)	60.4 (3.4)
♀ NAC	37.9 (0.7)	21.6 (3.1)

generation 11

★ indicates a difference significant at the .05 level.

Table 81

Variations of acetic acid larval preferences between progeny
of the reciprocal hybridizations between AC-line and NAC-line.
T-test and probability values p as in table 76.

variation between	P(generation 9)	P(generation 11)
♀ AC x ♂ NAC progeny		
and	< .01	< .01
♀ NAC x ♂ AC progeny		
variation between		
♀ AC x ♂ NAC progeny		
and	< .2	< .9
AC		
variation between		
♀ AC x ♂ NAC progeny		
and	< .01	< .01
NAC		
variation between		
♀ NAC x ♂ AC progeny		
and	< .001	< .01
AC		
variation between		
♀ NAC x ♂ AC progeny		
and	< .05	< .01
NAC		

Table 82

Percentages of males and females found among acetic acid and non acetic acid preferring larvae issued from AC-line x NAC-line reciprocal hybridizations at generation 11 of selection. Probability values p (that the observed percentages differ from 50 %) were determined by a standard normal table.

crossing pattern	% progeny larvae found in medium with acetic acid (I)		% progeny larvae found in medium without acetic acid (II)		Number of observations	P in I	P in II
	♂	♀	♂	♀			
♀ AC x ♂ NAC	51.8	48.2	52.0	48.0	185	∼.74	∼.69
♀ NAC x ♂ AC	50.5	49.5	44.9	55.1	203	∼.92	∼.31

Table 83

Mean egg-larval mortality and mortality of the pupal stage of AC-, ACT-, NAC- and NACT-lines calculated over the whole selection experiment.

selected lines	mean egg-larval mortality (%)	number of observations	mean mortality at the pupal stage (%)	number of observations
AC-line	6.7	3300	3.3	600
ACT-line	7.3	3300	3.5	600
NAC-line	4.1	3300	3.0	600
NACT-line	8.1	3300	4.2	600

CHAPTER 5 : ADDITIONAL DISCUSSION

5.1 - Introduction

By considering all the differences in preference between traumatic and rewarded lines reported in chapters 2, 3 and 4, the picture which emerges can be summarized as follows. In "two-way" selection for early preferences the divergence between up- and down-lines was larger when all selected individuals experienced the most "unpreferred" environmental conditions, as revealed by the genetic architecture of the trait under consideration. Thus, the greatest divergences were recorded between lines whose pupae and adults were exposed to dark for _D. simulans_ (D- and LT-lines), to light for _D. melanogaster_ (L- and DT-lines) and to atmospheres with ethanol (AL- and NALT-lines) but without acetic acid (ACT- and NAC-lines) for _D. melanogaster_ too. The possibility of an underlying "mechanism" behind this idea that adversity might favour diversity or genetic polymorphism in the wild, however, can by no means be discussed here, even though this may seem to be potentially relevant to Mayr's (1963) general statement that behavioural changes precede morphological changes during evolution.

In this last chapter I shall concentrate on
theoretical possibilities that could account for the
results relative to the discrepancies recorded between
rewarded and traumatic lines, since the other findings
have been discussed chapter by chapter. Before considering
possible explanations, one characteristic effect of the
traumas should be emphasized. This is the trend shown by
those traumatic lines that responded to selection to
evolve towards the fitter trait, that is to say the one
that was least responsive to conventional selection. In
D. simulans, the DT-line larvae thus preferred more light
PSs, whereas in D. melanogaster the DT-line larvae showed
more dark PSP. Similarly, in D. melanogaster both the ALT-
and NALT-line larvae tended to prefer media without alco-
hol and the NACT-line larvae tended to prefer media con-
taining acetic acid. Such a general trend for the trauma-
tic treatments to cause a shift towards an apparent in-
crease of the frequency of the fittest phenotypes must be
accepted with caution since only four comparisons have
been made. Nonetheless, it leads us to further consider
the possibility that intra-line natural selection was
operating in the traumatic lines.

5.2 - Possible explanations to account for the effects of the traumas

5.2.1 - The possibility of intra-line selection

Markow (1975c) observed that photopositive

strains of D. melanogaster selected in a phototactic maze
lay more eggs in continuous light than in a continuously
dark environment, while the opposite is true for photo-
negative strains. The author showed that absence of in-
semination was not likely to be the cause of the alter-
ation of oviposition rates, therefore the results reflect
the light dependent fitness of flies which is directly
related to their genotype for phototactic behaviour.

In my experiments, if the trauma selectively
affected less fit flies, making them lay fewer eggs, this
should have brought about a measurable reduction in fecund-
ity in traumatic lines. Viability effects would have
similarly increased mortality rates in some of the lines.
The results failed to support the view that the individual
parents contributed unequally (2.3.13) to the offspring
generations between the lines compared. Besides, such a
mechanism would not explain why traumatic lines can dis-
play mean phenotypic preferences that cannot be achieved
by conventional selection, as exemplified by D. melanogas-
ter D- and DT-lines. Furthermore, the chromosomal analysis
carried out between D2- and DT2-lines in this species
suggested that D- and DT-lines' second and third chromo-
somes were undistinguishable in their effects. As already
pointed out, assuming that there is a genetic correlation
between PSP and OSP (3.3.24 and 3.3.25), OSPs of the DT-
lines should also have been affected by the effect of the
trauma. This was not observed in D. simulans and apparent-

ly not in <u>D</u>. <u>melanogaster</u> either, which further militates against the possibility of intra-line selection.

5.2.2 - <u>The possibility of a purely environmental effect</u>

Effect of the environment on the development of the adult visual system in <u>Drosophila</u> is illustrated for instance by the rdgB mutants (Stark <u>et</u> <u>al</u>., 1983), which have hereditary retinal degeneration, dependent on both temperature and lighting. However, as reminded below, my results clearly demonstrated that the light induced change was stable over at least three generations in the absence of light and therefore cannot be assimilated to a similar environmental effect.

Falconer (1981) discusses the effects of selection on differences of environmental sensitivity and stresses that high sensitivity will be selected for when the selection and the environment act on the character in the same direction. Environments can indeed be referred to as "good" or "bad" according to whether they increase or decrease the character. However, by considering the experimental designs used in this thesis, one cannot argue that the increased divergence between upward and downward selected lines which was achieved through exposure of the pupae and young adults to "unpreferred" environments, resulted from a difference in environmental sensitivity of the flies compared. This is because, as already pointed

out, the environmental conditions were identical for all
lines until selection was performed so that no genotype-
environment interaction might have arisen from differences
in sensitivity to the environment.

As just mentioned,the possibility of a purely
environmental effect was ruled out by the observation
that the induced effect was stable when subD- and subDT-
lines were kept under uniform or reversed lighting con-
ditions (2.2.12, 3.2.7 and 3.2.8). The lack of effect with
inbred lines also failed to support a mere environmental
effect. In addition, the fact that the PL- and PD- mass
mated unselected lines showed stable PSPs (with perhaps
the exception of D. melanogaster PD-line) suggests that
the trauma itself might have represented the crucial event
through which the divergence was promoted.

5.2.3 - The possibility of an increase of the mutation
rate resulting from the trauma

This possibility seems unlikely as there is no
evidence that either visible light, ethanol or acetic acid
at the concentrations used, are effective mutagens. The
light intensity used as a traumatic treatment (236 F.C. at
the surface of the vials containing the pupae) was low if
one compares it to the light intensity of mountainous
regions for instance, where it can be more than forty

times as high on clear sunny days (Kekić and Marinković,
1974). As Dobzhansky (1970) emphasizes "the fact that the
frequencies of induced gene mutations are directly pro-
portional to the amounts of radiation administered" also
makes it unlikely that mutations were induced by the very
small amount of ionizing radiation present in the light
traumatic treatments,as the ultraviolet component was
probably very low anyway. Besides, the rapidity with which
DT-line PSPs diverged from D-line PSPs in D. simulans
implies that a substantial proportion of DT-line pupae
must have experienced such light-induced mutations in two
or three generations which is hard to believe with respect
to the weak dose of radiation applied. In any case, most
mutations are deleterious and would therefore be expected
to lower the general fitness of their bearers which was
clearly not observed, as just argued in 5.1.

It is still worth noticing that in Drosophila ad-
ults many mutations are known to affect the visual system
and Kourilsky and Gachelin (1984) report that mutations
found at the "white" locus, which modify the eye colour,
result from the action of a transposable element, which
apparently inactivates a gene involved in the eye pigment-
ation process.

If one assumes, however, that all that the
trauma did was to increase the mutation rate this should
have resulted in an increase of the response to the con-

ventional selection, which was not always observed. In D.
simulans DT-lines, where the effect of the trauma was
apparently more marked than in any of the other lines, the
opposite effect was observed, as the traumatic lines be-
came more light preferring. Similarly, if some other gene-
tic disorder such as hybrid dysgenesis had resulted from
the trauma, this would have increased the mutation rate
and could have caused sex ratio distortion (Bregliano and
Kidwell, 1983) but again the consequence of this should
have been an increase in response to conventional select-
ion.

5.2.4 - The possibility of a purely cytoplasmic effect

The possibility that some cytoplasmic factor
might have been somehow induced in the traumatic lines is
remote when one considers the results yielded by ID- and
IDT- isogenic lines. Thus, the lack of effect of the
trauma in IDT-line relative to DT-lines militates against
a cytoplasmic effect. Furthermore, in general there were
no consistent maternal effects in crosses between trauma-
tic and rewarded lines as would be expected if the cyto-
plasm alone was responsible for the findings.

However, some results cannot be explained by
sex-linkage alone and it was suggested that the environ-
ment experienced by the maternal ancestry might have con-
tributed to the change in preference. The first hint for

the possible involvement of a weak cytoplasmic effect in outbred lines was provided by the results of some crosses which suggested that when the cytoplasm of zygotes stemmed from flies which were repeatedly exposed to light, this might have altered the expression of the preferences in descendants. In D. melanogaster the view according to which such light induced effect could have enhanced the expression of the genes controlling dark PSP was partly supported by the results of the crosses using LT2-line flies (3.3.14 and 3.3.15), tester females (3.3.16) and particularly by the bias in sex ratio observed in DT-lines (3.3.20).

Whatever is the nature of the effect of trauma it is worth realizing that it must have operated during the early pupal stage (when the trauma was applied). Interestingly, this period is close to the late larval period during which D. melanogaster has been observed to be subject to dauermodifications (Jollos, 1935), in which cytoplasmic inheritance was almost certainly involved (see 1.10). Although the possibility of a similar cytoplasmic effect was not substantiated by the data, the possibility of an interaction between nucleus and cytoplasm will be further considered at this point, as on theoretical grounds this seems to remain a possible explanation that ought to be discussed.

5.2.5 - <u>The possibility of a nucleus-cytoplasm interaction</u>

Genotypic effects may be considerably modified by environment and Strickberger (1976) stressed that "in the cell itself one important source of environmental effect is the cytoplasm immediately surrounding the nucleus". It would then hardly be surprising to find that the same genotype would function somewhat differently when placed in different cytoplasms. As some results reported in the present thesis pointed to the possibility that the control of the traits studied might not be restricted to nuclear genes alone, one can suppose that the trauma somehow affected first the cytoplasmic state, which might then have affected the norm of reaction of the nuclear genome. As Dobzhansky (1970) points out "the norm of reaction is the entire range, the whole repertoire, of the variant paths of development that may occur in the carriers of a given genotype in all environments, favourable and unfavourable, natural and artificial".

In view of the lack of simple explanation for the effects of the traumas, I would be prepared to consider a hypothesis which emphasizes a nucleus-cytoplasm interaction in the following way. Consistent exposure of individuals to "unpreferred" environmental conditions might under certain circumstances affect a cytoplasmic component, ultimately enhancing the expression of some of the nuclear genes involved in the control of the preference for the environ-

mental variable under consideration. This hypothesis would then be supported by the observation that more between-line difference in preference was always achieved when bi-directional selection was performed under the most "un-preferred" environmental conditions, as revealed by the genetic architecture of the trait studied.

Future experiments should further investigate this possibility and try to standardize contribution from individuals to fully control for any effect of intra-line selection.

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LITERATURE CITED

Aceves-Piña, E.O. & Quinn, W.G. (1979). Learning in normal and mutant Drosophila larvae. Science 206 : 93-96.

Atkinson, W. & Shorrocks, B. (1977). Breeding site specificity in the domestic species of Drosophila. Oecologia 29 : 223-232.

Basden, E.B. (1954). The distribution and biology of Drosophilidae (Diptera) in Scotland, including a new species of Drosophila. Transactions of the Royal Society of Edinburgh 62 : 602-654.

Benzer, S. (1967). Behavioral mutants of Drosophila isolated by countercurrent distribution. Proc. Natl. Acad. Sci. U.S.A. 58 : 1112-1119.

Benzer, S. (1973). The genetic dissection of behavior. Scientific American 229 : 24-37.

Bos, M. & Boerema, A.C. (1980). Oviposition preferences in six Drosophila melanogaster-subgroup species. Drosophila Information Service 55 : 15-16.

Bregliano, J.C. & Kidwell, M.G. (1983). Hybrid dysgenesis determinants. In : "Mobile Genetic Elements" (ed. J. S. Shapiro). Academic Press, New York.

Brown, F.A. & Hall, B.V. (1936). The directive influence of light upon Drosophila melanogaster Meig. and some of its eye mutants. Journal of Experimental Zoology 74 : 205-220.

Bush, G. L. (1975). Modes of animal speciation. Ann. Rev. Ecol. Syst. 6 : 339-364.

Carson, H.L. (1971). The ecology of Drosophila breeding sites. In : "Harold L. Lyon Arboretum Lecture" 2 : 1-27. Honolulu. University of Hawaï.

Clausen, J., Keck, D.D. & Hiesey, W.M. (1940). Experimental studies on the nature of species. I. Effects of varied environments on western North American plants. Carnegie Inst. of Washington, Publ. 520 : 1-452.

David, J., Fouillet, P. & Arens, M.F. (1974). Comparaison de la sensibilité à l'alcool éthylique des six espèces de Drosophila du sous-groupe melanogaster. Arch. Zool. exp. gén., 115 : 401-410.

Del Solar, E. (1968). Selection for and against gregariousness in the choice of oviposition sites by Drosophila pseudoobscura. Genetics 58 : 275-282.

Demerec, M. (Ed.) (1950). The Biology of Drosophila. New York , Hafner.

Dethier, V.G. (1954). Evolution of feeding preferences in phytophagous insects. Evolution 8 : 33-54.

Dobzhansky, T. (1970). Genetics of the Evolutionary Process. Columbia University Press.

Dobzhansky, T. & Spassky, B. (1967). Responses of various strains of Drosophila pseudoobscura and Drosophila persimilis to light and gravity. Amer. Natur. 101 : 59-63.

Dobzhansky, T. & Spassky, B. (1969). Artificial and natural selection for two behavioral traits in Drosophila pseudoobscura. Proc. Natl. Acad. Sci. 62 : 75-80.

Falconer, D.S. (1981). Introduction to Quantitative Genetics. 2nd Edition, Longman, London.

Felsenstein, J. (1976). The theoretical population genetics of variable selection and migration. Ann. Rev. Genet. 10 : 253-280.

Fisher, R.A. (1930). The Genetical Theory of Natural Selection. Clarendon Press, Oxford.

Fraenkel, G.S. & Gunn, D.L. (1940). The orientation of animals. Oxford University Press.

Fuller, J.L. & Thompson, W.R. (1978). Foundations of Behavior Genetics. C.V. Mosby, St. Louis, Missouri.

Futuyma, D.J. & Mayer, G.C. (1980). Non-allopatric speciation in animals. Syst. Zool. 29 : 254-271.

Fuyama, Y. (1976). Behavior genetics of olfactory responses in Drosophila. I. Olfactometry and strain differences in Drosophila melanogaster. Behav. Genet. 6 : 407-420.

Gelfand, L.J. & McDonald, J.F. (1983). Relationship between alcohol dehydrogenase (ADH) activity and behavioral response to environmental alcohol in five Drosophila species. Behav. Genetics 13 : 281-293.

Gordon, C. & Sang, J.H. (1942). Polygenic inheritance and the Drosophila culture. Nature 149 : 610-611.

Gould, J.L. & Gould, C.G. (1983). Can a bee behave intelligently? New Scientist 98 : 84-87.

Grossfield, J. (1966). The influence of light on the mating behavior of Drosophila. Stud. Genet. (Univ. Tex. Publ. 6615) 3 : 147-176.

Grossfield, J. (1968). The relative importance of wing utilization in light dependent courtship in Drosophila. Stud. Genet. (Univ. Tex. Publ. 6818) 4 : 147-156.

Grossfield, J. (1970). Evidence for the genetic control of a complex behavior in Drosophila. Genetics 64 : s27.

Grossfield, J. (1971). Geographic distribution and light dependent behavior in Drosophila. Proc. Natl. Acad. Sci. 68 : 2669-2673.

Grun, P. (1976). Cytoplasmic Genetics and Evolution. Columbia University Press, New Nork.

Guttman, S.I., Wood, T.K. & Karlin, A.A. (1981). Genetic differentiation along host plant lines in the sympatric Enchenopa binotata Say complex (Homoptera: membracidae). Evolution 35 : 205-217.

Hadler, N. (1964a). Genetic influence on phototaxis in

Drosophila melanogaster. Biol. Bull. 126 : 264-273.

Hadler, N. (1964b). Heritability and phototaxis in Drosophila melanogaster. Genetics 50 : 1269-1277.

Hanson, T.E. (1978). Neurogenesis in the lamina of the Drosophila visual system. I. Cortical interactions - optic cartridge and twist development (unpublished manuscript).

Hirsch, J. (1967). Behavior-genetic analysis at the chromosome level of organization. In : "Behavior-genetic analysis". J.Hirsch, ed. McGraw-Hill Book Company, New York.

Hoenigsberg, H.F. (1968). An ecological situation which produced a change in the proportion of Drosophila melanogaster to D. simulans. Amer. Natur. 102 : 389-390.

Hopkins, A.D. (1917). A discussion of C.G. Hewitt's paper on "Insect Behaviour". J. Econ. Ent. 10 : 92-93.

Hotta, Y. & Benzer, S. (1970). Genetic dissection of the Drosophila nervous system by means of mosaics. Proc. Natl. Acad. Sci. U.S.A. 67 : 1156-1163.

Hotta, Y. & Benzer, S. (1972). The mapping of behavior in Drosophila mosaics. Nature 240 : 527-535.

Jollos, V. (1935). Studien zum Evolutionsproblem. II.

Dauermodifikationen, "plasmatische Vererbung" und ihre Bedeutung für die Entstehung der Arten. Biol. Zbl. 55 : 390-436.

Jones, J.S. (1980). Can genes choose habitats? Nature 286 : 757-758.

Jones, J.S. (1982). Genetic differences in individual behaviour associated with shell polymorphism in the snail Cepaea nemoralis. Nature 298 : 749-750.

Jungen, H. & Wunderlich, E. (1972). Abhängigkeit des Geschlechtsverhältnisses vom Elternalter in sr-Stämmen von Drosophila subobscura. Archiv für Genetik 45 : 192.

Kankel, D.R., Ferrús, A., Garen, S.H., Harte, P.J. & Lewis, P.E. (1980). The structure and development of the nervous system.In:"The Genetics and Biology of Drosophila", vol. 2 d (eds Ashburner and Wright). Academic Press, New York.

Kawanishi, M. & Watanabe, T.K. (1978). Difference in photo-preferences as a cause of coexistence of Drosophila simulans and Drosophila melanogaster in nature . Japan.J. Genetics 53 : 209-214.

Kekić, V. & Marinković, D. (1974). Multiple-choice selection for light preference in Drosophila subobscura. Behav. Genet. 4 : 285-300.

Kekić, V., Taylor, C.E. & Andjelković, M. (1980). Habitat choice and resource specialization by Drosophila subobscura. Genetika 12 : 219-225.

Kourilsky, P. & Gachelin, G. (1984). L'organisation de
        l'information génétique. La Recherche 155 : 642-651.

McDonald, J. & Parsons, P.A. (1973). Dispersal activities
        of the sibling species <u>Drosophila</u> <u>melanogaster</u> and
        <u>Drosophila</u> <u>simulans</u>. Behav. Genet. 3 : 293-301.

Mackay, T.F.C. (1981). Genetic variation in varying en-
        vironments. Genet. Res. 37 : 79-93.

McKenzie,J.A. & McKechnie S.W. (1978). Ethanol tolerance
        and the Adh polymorphism in a natural population
        of <u>Drosophila</u> <u>melanogaster</u>. Nature 272 : 75-76.

McKenzie, J.A. & McKechnie, S.W. (1979). A comparative
        study of resource utilization in natural popul-
        ations of <u>Drosophila</u> <u>melanogaster</u> and <u>D</u>. <u>simulans</u>.
        Oecologia 40 : 299-309.

McKenzie, J.A. & Parsons, P.A. (1972). Alcohol tolerance :
        an ecological parameter in the relative success of
        <u>Drosophila</u> <u>melanogaster</u> and <u>Drosophila</u> <u>simulans</u>.
        Oecologia 10 : 373-388.

McKenzie, J.A. & Parsons, P.A. (1974). Microdifferentiation

in a natural population of <u>Drosophila</u> <u>melanogaster</u>
to alcohol in the environment. Genetics 77 : 385-394.

Manning, A. (1967)."Pre-imaginal conditioning" in <u>Droso</u>-
<u>phila</u>. Nature 216 : 338-340.

Manning, M. & Markow, T.A. (1981). Light-dependent pup-
ation site preferences in <u>Drosophila</u>. II. <u>Drosophi</u>-
<u>la</u> <u>melanogaster</u> and <u>Drosophila</u> <u>simulans</u>. Behav.
Genet. 11 : 557-563.

Markow, T.A. (1975a). A genetic analysis of phototactic
behavior in <u>Drosophila</u> <u>melanogaster</u>. I. Selection
in the presence of inversions. Genetics 79 : 527-
534.

Markow, T.A. (1975b). A genetic analysis of phototactic
behavior in <u>Drosophila</u> <u>melanogaster</u>. II. Hybridiz-
ations of divergent populations. Behav. Genet. 5 :
339-350.

Markow, T.A. (1975c). Effect of light on egg-laying rate
and mating speed in phototactic strains of <u>Droso</u>-
<u>phila</u>. Nature 258 : 712-714.

Markow, T.A. (1981). Light-dependent pupation site prefer-
ences in <u>Drosophila</u> : behavior of adult visual
mutants. Behavioral and Neural Biology 31 : 348-353.

Markow, T.A. & Smith, L.D. (1979). Genetics of phototactic
behavior in <u>Drosophila</u> <u>ananassae</u>, a member of the
<u>melanogaster</u> species group. Behav. Genet. 9 : 61-
67.

Markow, T.A. & Smith, W.L. (1977). Genetic analysis of

phototactic behavior in Drosophila simulans.
Genetics 85 : 273-278.

Maynard Smith, J. (1966). Sympatric speciation. Amer.
Natur. 100 : 637-650.

Mayr, E. (1947). Ecological factors in speciation.
Evolution 1 : 263-288.

Mayr, E. (1963). Animal Species and Evolution. Harvard
University Press, Cambridge, Massachusetts.

Mayr, E. (1978). (Review of) Modes of speciation. Syst.
Zool. 27 : 478-482.

Médioni, J. (1962). Contribution à l'étude psycho-physio-
logique et génétique du phototropisme d'un insecte
Drosophila melanogaster Meigen. Thesis. University
of Strasbourg.

Okada, T. (1971). Taxonomic and ecological notes on the
Drosophilidae of Bonin Islands (DIPTERA). Proc. Jap.
Soc. Syst. Zool. 7 : 67-73.

Parsons, P.A. (1975). Phototactic responses along a
gradient of light intensities for the sibling
species Drosophila melanogaster and Drosophila si-
mulans. Behav. Genet. 5 : 17-25.

Parsons, P.A. (1977). Larval reaction to alcohol as an
indicator of resource utilization differences
between Drosophila melanogaster and D. simulans.
Oecologia 30 : 141-146.

Parsons, P.A. (1978). Habitat selection and evolutionary
strategies in Drosophila : An Invited Address.

Behavior Genetics 8 : 511-526.

Parsons, P.A. (1979). Larval reactions to possible resources in three species of Drosophila as indicators of ecological diversity. Aust. J. Zool. 27 : 413-419.

Parsons, P.A. & Spence, G.E. (1981). Longevity, resource utilization and larval preferences in Drosophila : inter- and intraspecific variation. Aust. J. Zool. 29 : 671-678.

Parsons, P.A., Stanley, S.M. & Spence, G.E. (1979). Environmental ethanol at low concentrations : longevity and development in the sibling species Drosophila melanogaster and D. simulans. Aust. J. Zool. 27 : 747-754.

Partridge, L. (1978). Habitat Selection. In : "Behavioural Ecology, an evolutionary approach." ed. by J.R. Krebs & N.B. Davies. Blackwell Scientific Publications : 351-376.

Pimentel, D., Smith, G.J.C. & Soans, J. (1967). A population model of sympatric speciation. Amer. Natur. 101 : 493-504.

Polivanov, S. (1975). Response of Drosophila persimilis to phototactic and geotactic selection. Behav. Genet.5: 255-258.

Roberts, M.B.V. (1971). Biology, A Functional Approach. Thomas Nelson & Sons Ltd, London.

Rockwell, R.F. & Seiger, M.B. (1973). Phototaxis in

<u>Drosophila</u> : a critical evaluation. Amer. Sci. 61 : 339-345.

Sameoto, D.D. & Miller, R.S. (1968). Selection of pupation site by <u>Drosophila</u> <u>melanogaster</u> and <u>Drosophila</u> <u>simulans</u>. Ecology 49 : 177-180.

Scott, J.P. (1943). Effects of single genes on the behavior of <u>Drosophila</u>. American Naturalist 77: 184-190.

Sharp, P.M. (1982). Quantitative genetics of <u>Drosophila</u> <u>melanogaster</u> - variation in male mating ability. Ph.D. thesis, Edinburgh University.

Sokal, R.R. (1966). Pupation site differences in <u>Drosophila</u> <u>melanogaster</u>. The University of Kansas science bulletin XLVI : 697-715.

Sokal, R.R., Ehrlich, P.R., Hunter, P.E. & Schlager, G. (1960). Some factors affecting pupation site of <u>Drosophila</u>. Ann. Entomol. Soc. Amer. 53 : 174-182.

Sokolowski, M.B. & Hansell, R.I.C. (1983). <u>Drosophila</u> larval foraging behavior. I. The sibling species, <u>Drosophila</u> <u>melanogaster</u> and <u>D</u>. <u>simulans</u>. Behav. Genet. 13 : 159-168.

Spieth, H.T. & Hsu, T.C. (1950). The influence of light on seven species of the <u>Drosophila</u> <u>melanogaster</u> species group. Evolution 4 : 316-325.

Stark, W.S., Chen, D., Johnson, M.A. & Frayer, K.L. (1983). The rdgB gene in <u>Drosophila</u> : retinal degeneration

in different mutant alleles and inhibition of degeneration by norpA. J. Insect. Physiol. 29 : 123-131.

Starmer, W.T., Heed, W.B. & Rockwood-Sluss, E.S. (1977). Extension of longevity in Drosophila mojavensis by environmental ethanol : differences between subraces. Proc. Natl. Acad. Sci. 74 : 387-391.

Strickberger, M.W. (1976). Genetics. 2nd edition, Macmillan Publishing Co., INC. New-York.

Tantawy, A.O., Mourad, A.M. & Masry, A.M. (1970). Studies on natural populations of Drosophila. VIII. A note on the directional changes over a long period of time in the structure of Drosophila near Alexandria, Egypt. Amer. Natur. 104 : 105-109.

Tauber, C.A. & Tauber, M.J. (1977). A genetic model for sympatric speciation through habitat diversification and seasonal isolation. Nature 268 : 702-705.

Taylor, C.E. (1976). Genetic variation in heterogeneous environments. Genetics 83 : 887-894.

Taylor, C.E. & Powell, J.R. (1978). Habitat choice in natural populations of Drosophila. Oecologica 37 : 69-75.

Thomas, J.B. & Wyman, R.J. (1982). A mutation in Drosophila alters normal connectivity between two identified neurones. Nature 298 : 650-651.

Thorpe, W.H. (1938). Further experiments on olfactory conditioning in a parasitic insect. The nature of the conditioning process. Proc. Roy. Soc. B, 126 :

370-397.

Truman, J.W. (1976). Extraretinal photoreception in insects. Photochemistry and Photobiology 23 : 215-225.

Van Herrewege, J. & David, J.R. (1978). Feeding an insect through its respiration : assimilation of alcohol vapors by Drosophila melanogaster adults. Experientia 34 : 163-164.

Vaysse, G. & Médioni, J. (1982). L'emprise des gènes et les modulations expérientielles du comportement. Bios. Université Paul Sabatier. Privat.

Waddington, C.H. & Perry, M.M. (1960). The ultra-structure of the developing eye of Drosophila. Proc. Roy. Soc. B, 153 : 155-178.

Waddington, C.H., Woolf, B. & Perry, M.M. (1954). Environment selection by Drosophila mutants. Evolution 8 : 89-96.

Wallace, B., & Dobzhansky, T. (1946). Experiments on sexual isolation in Drosophila. VIII. Influence of light on the mating behavior of Drosophila sub-obscura, Drosophila persimilis and Drosophila pseudoobscura. Proc. Natl. Acad. Sci. 32 : 226-234.

Walton, P.D. (1970). The genetics of phototaxis in Drosophila melanogaster. Can. J. Genet. Cytol. 12 : 283-287.

Wasserman, S.S. & Futuyma, D.J. (1981). Evolution of host plant utilization in laboratory populations of the

southern cowpea weevil, Callosobruchus maculatus
Fabricius (Coleoptera : Bruchidae). Evolution 35 :
605-617.

Watanabe, T.K. & Kawanishi, M. (1976). Colonization of
Drosophila simulans in Japan. Proc. Jap. Acad. 52 :
191-194.

White, K. & Kankel, D.R. (1978). Patterns of cell division
and cell movement in the formation of the imaginal
nervous system in Drosophila melanogaster. Devl.
Biol. 65 : 296-321.

White, M.J.D. (1978). Modes of speciations. W.H. Freeman,
San Francicso : 455 pp.

Williams, G.C. (1974). Adaptation and Natural Selection.
Princeton University Press, Princeton, New Jersey.

Wood, T.K. (1980). Divergence in the Enchenopa binotata
Say complex (Homoptera : Membracidae) effected by
host plant adaptation. Evolution 34 : 147-160.

Woolf, C.M. (1972). Genetic analysis of geotactic and
phototactic behavior in selected strains of Droso-
phila pseudoobscura. Behav. Genet. 2 : 93-106.

Wright, S. & Dobzhansky, Th. (1946). Genetics of natural
populations. XII. Experimental reproduction of some
of the changes caused by natural selection in
certain populations of Drosophila pseudoobscura.
Genetics 31 : 125-156.