Chapter 1

Introduction

1.1 Aim of Project

A life-history strategy is the pattern of survivorship and fecundity observed in a natural population (Hedrick 1984). It is comprised of a number of components termed life-history traits or fitness components, such as clutch size, age at maturity and length of reproductive life-span, which together, constitute the total life-history strategy (Wilbur, Tinkle and Collins 1974). Life-history strategies have been increasingly viewed as adaptive responses to environmental variables and demographic constraints (Cole 1954; Lack 1954a; Stearns 1983a; Sutherland, Grafen and Harvey 1986; Williams 1966a), which are supposed to push a population towards an optimal combination of life-history traits (Stearns 1976).

The evolution of life-histories must be subject to internal constraints; otherwise one would expect all organisms to reach reproductive maturity early, to produce many young and to attain immortality. The general aim of this project is to investigate the importance and nature of the genetic constraints.

Some theory proposes that the fundamental constraint on life-history evolution is a trade-off between early and late reproduction and/or age at death due to the optimal allocation of the limited resources available to the organism. It is one objective of this project to determine whether such trade-offs exist. This will be achieved by the examination of the genetic correlations between early and late reproduction and age at death in a laboratory population recently derived from the wild.

An adjunct to this study is to identify the forces which maintain senescence as an almost universal property of living organisms. Although average age at death is probably subject to

natural selection, many different hypotheses have been proposed to explain why senescence itself, a deleterious trait at the level of the individual, has not been eliminated by natural selection. Senescence is possibly the ultimate constraint on the fitness of an individual and the evolution of life-histories.

Further constraints on life-history evolution may be the extent and nature of genetic variation and covariation of life-history traits. It is a matter of debate how much additive genetic variation exists for fitness components in natural populations, how such variation is maintained and the extent to which genetic correlations with other traits limit the responses of life-history traits to natural selection.

The following sections of this chapter review theoretical and empirical work which has attempted to elucidate the genetic basis of life-history evolution.

1.2 The Cost of Reproduction

For organisms with non-overlapping generations, the fitness of an individual is the contribution of genes that it makes to the next generation, or the number of its progeny represented in the next generation (Falconer 1981). However, with overlapping generations fitness amounts to much more than the contribution of genes to the next generation. In either case, fitness has to be a function of the life-history strategy of the individual (Templeton and Johnston 1982), and the fitness of an individual depends not only on the total quantity of its reproduction but on its value, which, amongst other factors, is determined by the individual's schedule of reproduction (Ohba 1967), the age-structure of the population to which it belongs (Charlesworth 1980) and its survival probability. Natural selection doesn't always select those indivduals that have on the average more offspring; it also acts to decrease variances in offspring number (Gillespie 1977). Thus fitness is dependent on the distribution of reproduction throughout the life-span, that is the age-specific viabilities and fecundities (Bell 1980; Templeton and Johnston 1982).

The relative importance of traits varies (Mertz 1971), but those which are considered to be important components of any life-history strategy are:

1) age at first reproduction;

2) length of reproductive life-span;

3) total number of eggs or young produced;

4) quality and/or size of eggs or young;

5) number of clutches per lifetime;

6) inter-brood interval; and

7) mortality schedule of juveniles and adults (Stearns 1976; Wilbur *et al.* 1974).

These are all measures in some respect of the quality and/or quantity of offspring produced. Net fitness is not necessarily highly correlated with any individual component (Yamazaki and Hirose 1984) but is best estimated by combining the values of components.

Of course, life-history patterns also are determined by adaptations, such as dormancy, diapause or migration, which improve the survival and/or fecundity probability of an organism (Hedrick 1984). Their contributions to fitness are hard to quantify and thus are often ignored in analyses of maximisation of fitness.

It has been hypothesised that animals must have finite resources because their resource acquiring mechanisms are restricted and limiting (Calow 1984). Thus, an organism has finite energy reserves which must be divided between maintenance, growth and reproduction. Natural selection might be expected to achieve an optimal allocation of resources among and within these to optimise reproductive output (Gadgil and Bossert 1970). Thus the life-history strategy of any species may be indicative of an optimal allocation of energy, and the attainment of maximum fitness within these energetic constraints (Benton and Uetz 1986).

An optimum allocation of finite energy stores therefore implies that allocation to any one of maintenance, growth and reproduction means a reduction (i.e. cost) to the others. Given this concept, the theory of the cost of reproduction has developed. This theory is the basis upon which all postulated models of life-history evolution depend (e.g. Caswell 1982a,b; Charlesworth and León 1976; Fagen 1972; Gadgil and Bossert 1970; Goodman 1974; León 1976; Michod 1979; Schaffer and Gadgil 1975; Schaffer and Rosenzweig 1977; Taylor, Gourley, Lawrence and Kaplan 1974). Bell (1984a) goes so far as to say that "it is not possible to optimise the life-history unless some cost of reproduction exists".

1.2.1 The Theorem of Reproductive Cost

Costs of reproduction have traditionally been seen as due to the diversion of energy away from the soma into reproduction. Williams (1966a,b) was the first to hypothesise that

an increase in present reproduction would result in decreased future reproduction, because either future survival or future fecundity would be reduced. This would be because resources would have to be diverted away from somatic investment into reproduction to increase the output. The reduced somatic investment would deleteriously affect important physiological processes of the organism and also reduce its ability to escape predators or forage for food (Calow 1984). Thus there could be "survival costs" for the parent. Future reproduction also could be affected, as energy would be directly diverted from it to boost present reproduction: "fecundity costs" (Tuomi, Hakala and Haukioja 1983).

Gadgil and Bossert (1970) assume that an increase in resources to reproduction would also affect growth detrimentally, which may reduce the potential for reproduction later in life, particularly for organisms of indeterminate size. A negative relationship between reproduction early in life and the chances of survival and/or reproduction later in life demonstrate the existence of a reproductive cost (Law 1979).

It is obvious that the cost of reproduction has two components. One is a direct decrease in the organism's future survival and/or fecundity because energy requirements are not met (Calow 1979; Reznick 1983) and is independent of the environment. The other component results from interaction with the environment, where, by the increasing diversion of energy to reproduction, the organism is made more vulnerable to its habitat (Calow 1979; Gremare and Olive 1986; Haukioja and Hakala 1979; Hirshfield 1980; Olive 1986; Reznick 1983; Shine 1980; Vitt and Congdon 1978). This second type of reproductive cost is not easily measured within the laboratory situation of unlimited resources, though attempts have been made to measure the effects of predation on gravid *Daphnia pulex* (Koufopanou and Bell 1984).

Fisher (1930) first pointed out that the pattern of energy allocation between reproductive tissues and the soma was probably responsible for the moulding of life-history strategies of reproduction. Williams (1966a,b) suggested that demographic forces selected the optimal pattern of energy allocation for a particular population within a specific environment. The trade-off between present and future reproduction and/or survival leads organisms to maximise their fitness by practising some optimal compromise between present and future reproductive rates. It was originally assumed that a measure of energy allocated to reproduction is a measure of the cost of reproduction for the individual.

Reproductive effort, E(x), is defined as the percentage of the resources of an organism, aged x, that are directly invested in reproduction (Hirshfield and Tinkle 1975; Charlesworth 1980). On the basis of energy allocation principles, survivorship to age x+1, P(x), and

fecundity at age x, m(x), are decreasing and increasing functions of E(x) respectively (Gadgil and Bossert 1970; Pianka and Parker 1975; Schaffer 1974b; Williams 1966b).

The problem in empirical testing of the relationships between E(x), m(x) and P(x) is the measurement of reproductive effort. Most studies use the energetic costs of breeding, measured in terms of the number, weight, caloric value of eggs/young, or ratio of energetic costs of breeding as a fraction of the mother's total body weight or calorific value, as a measure of reproductive effort (e.g. Barber and Blake 1986; Calow 1979; Gremare 1986; Gremare and Olive 1986; Pianka and Parker 1975; Stearns 1976; Tinkle and Hadley 1975). However, the amount of energy devoted to reproduction does not necessarily measure the reproductive costs. Energy devoted to reproduction may not always be at the expense of somatic investment and thus there may not be any reproductive costs (Bell 1980; Clutton-Brock 1984; Tuomi, Hakala and Haukioja 1983). However, the reproductive effort theorem assumes that any increase in reproductive energy allotment is always at the expense of future survivorship or reproduction.

Also, when comparing reproductive effort in groups of organisms, the proportion of total energy allocated to reproduction only measures reproductive cost differences if the intake of resources is the same in the groups under comparison (Huakioja and Hakala 1978). There is always the possibility of increased resource uptake during breeding (Collatz and Wilps 1986; Hirshfield and Tinkle 1975), or, of drawing upon reserves stored when resources are plentiful (Tuomi *et al.* 1983). The adults of a variety of organisms store energy and nutrients well in advance of the reproductive season (see Reznick 1985 for examples). Van Noordwijk and De Jong (1986) have suggested that variation in resource allocation can best be demonstrated when the variation in resource acquisition is relatively small, while the average resource acquisition is high.

Another consideration is that the size of the individual is probably important in determining how costly a given energetic measure of reproductive effort is to its future survival and breeding potential (Clutton-Brock 1984).

Only increased energy which involves a trade-off with other traits should be regarded as a reproductive cost (Gremare and Olive 1986).

Bell (1980) would discard the concept of reproductive effort altogether in relation to the evolution of life-histories and the interpretation of age-specific schedules of reproduction.

Bell (1980,1984a) has given the cost of reproduction a broader definition: it is any decrement in the expectation of future reproduction because either future survival or fecundity

is reduced, due to an increment in present reproduction. This definition embraces the energetic concept of cost, but also includes costs whose causation is not easily related to a decrement in energy allocation. An example of such a cost may be the increasing vulnerability to predation the greater the number of unborn young carried by a pregnant female. Since this second type of cost could also be an important force moulding life-history evolution, it seems unwise to ignore its existence. For this reason alone I believe Bell's (1980,1984a) definition is more direct, less ambiguous and more appropriate than one based purely on energy allocation.

Theoretically reproductive cost is not a sufficient condition for the evolution of intermediate levels of reproduction, since some cost curves favour the greatest possible rate of reproduction or of survival (Bell 1984a). However, reproductive cost is a necessary condition for constraints on the evolution of life-histories. Its existence does need to be unrefutably established.

There has been some theoretical (Tuomi *et al.* 1983) and empirical work (e.g. Bell 1984a,b) that disputes the existence of such costs of reproduction.

Tuomi *et al.* (1983) have suggested that the numerous examples of organisms using various mechanisms to minimise costs of reproduction is argument against the universality of the theorem that reproductive costs mould life-histories. There are many examples of increased resource uptake during breeding (Hirshfield and Tinkle 1975), and of drawing upon reserves stored when resources are plentiful (Tuomi *et al.* 1983; and see Reznick 1985 for examples). There are also a number of studies that demonstrate that organisms can regulate their level of reproductive output depending on resource availability (Lack 1966; Low 1978; Merson and Kirkpatrick 1981; Morton, Recher, Thompson and Braithwaite 1982; Sota 1984), or even the likelihood of the survival of their progeny (Ekman and Askenmo 1986). Also, timing of reproduction can be crucial in minimising costs (Haukioja and Hakala 1979; Meagher and Antonovics 1982; Wallinga and Bakker 1978). Such regulation and prevention of energy wastage probably reduces the costs of reproduction to the parent. Some experimental manipulation studies show that the costs of reproduction are minimised or nonexistent in non-resource limiting, non-stressful environments (Boyer 1978; Browne 1982; Feifarek, Wyngaard and Allen 1983; Haukioja and Hakala 1978).

However, as Reznick (1985) states, "these mechanisms are all expressed in a fashion that is potentially independent of genetically based trade-offs". All of the above studies are based on phenotypic correlations, average characteristics of whole populations or experimental

manipulations. Thus it is possible that genetic variation for these costs is present within groups. The existence of mechanisms to avoid cost is suggestive in itself that reproductive costs may be present. Since these mechanisms could obscure the presence of reproductive costs, it is obvious that experiments that do not measure the genetic correlations between early and late fecundity may be misleading in their assessments.

I propose firstly to state what conditions need to be met to provide evidence for a trade-off between present fecundity and future survival and/or fecundity. Secondly, I propose to examine what type of experimental design fulfills these conditions; and thirdly, to appraise the current status of empirical work.

1.2.1.1 Conditions of Proof

It is necessary to establish that the "trade-offs" of reproduction have a genetic basis, otherwise they could have no evolutionary consequences (Charlesworth 1984; Law, Bradshaw and Putwain 1977; Reznick 1985). If correlations are purely phenotypic then there could be no adjustments in age-specific reproduction in response to selection pressures. Reproduction would just be maximised at all ages. Thus, to confirm the theory, there should be, within populations, negative genetic correlations between early and late fecundity, and/or between fecundity and survival (Reznick 1985). Since life-history strategies have been shown to be discernable at the intraspecific level (Brown 1983; Dunham and Miles 1985) and are adaptations to local environmental conditions (e.g. Reznick 1982), reproductive costs must exist at the population level if they are important in moulding age-specific reproductive schedules.

However, what does a negative genetic correlation between early and late fecundity show? A group of individuals in a population could produce more eggs per day throughout their life-spans than another group of individuals of equivalent life-span, but have a greater rate of decline of egg production. Bell's (1980,1984a) definition, strictly interpreted, would define the first group as exhibiting reproductive cost, but has such a "reproductive cost" any evolutionary consequences. The individuals which produce more at every point in their life-spans would always be favoured by natural selection, if reproduction is the only criterion, which in this case we assume it is. Within a population then, a change in ranking of individuals for early and late life fecundity must be established to demonstrate a fecundity cost. A negative genetic correlation demonstrates such a change in ranking.

Springer and Boggs (1986) have shown in *Colias philodice eriphyle* (Lepidoptera) and

Hughes and Hughes (1986) in *Celleporella hyalina* (Bryozoa), that resource allocation between the soma and reproduction has a genetic basis. This is only suggestive, not proof, that current energy allocation has implications for future fecundity and survival.

As proof of cost, a direct causal relationship should be established between present and future reproduction and survival (Bell 1984a). Bell (1984a) discounts any experimental work demonstrating a negative genetic relationship between reproduction and growth, as being only suggestive of a decreased future fecundity, unless a decrease in growth can be proven to have a direct effect on future mortality and fecundity. Bell (1984a) and Reznick (1985) also both emphasise that when using the environment to manipulate reproduction, one may be assuming that the responses of later reproduction and survival are direct consequences of the altered reproductive schedule, whereas in fact their responses are due to the changed environmental variable.

It is also not enough to establish a negative relationship between survival and reproduction per se; there must be a scaling effect. That is, with each increment in increased present reproductive output, there must be a corresponding decrement in future reproductive output and/or a decreasing probability of survival. Since it is necessary to reproduce, any damage resulting from processes of reproduction which affect individuals at random and which cannot be avoided, is not important in the evolution of life-histories. An example of this are the costs involved in mating (Daly 1978). A sexual organism must mate in order to leave offspring. Any possibility of physiological damage then, accruing from the mating ritual or the actual physical process of mating which decreases future survival and/or reproduction, is a risk which an organism must take. Only if it can be proven that organisms genetically vary in their susceptibility to damage, can it be termed a cost of reproduction as it is defined here.

Comparisons of genetic correlations between characters across species should not be done to determine if reproductive costs are a universal phenomenon. Trade-offs may differ between species. Certainly, genetic correlations between characters do differ for across and within species' comparisons (Bell 1984b), emphasising the need to use intraspecific comparisons of genetic correlations.

Furthermore, the existence of negative genetic correlations in a laboratory population does not conclusively prove that reproductive costs are important in the evolution of age-specific fecundities in a natural population. Negative genetic correlations between traits are not unexpected in laboratory populations, which experience consistent unidirectional

selection pressures (Falconer 1952; Lande 1980; Lerner 1950). It is important that negative genetic correlations between early and late fecundity and/or longevity be established for individuals of natural populations or for laboratory populations recently derived from the wild.

Any experimental work must abide by all of these above conditions before it can be judged to be producing valid estimates of the cost of reproduction.

1.2.1.2 Experimental Design

Reznick (1985) has comprehensively reviewed papers published prior to 1983 which are empirical studies of the cost of reproduction. He excluded a number of studies on the basis of poor experimental design; measurement of variables that are not comparable with those of other studies; poor experimental techniques; or on the basis that the results cannot be easily interpreted as measures of cost. He then classifies, by methodology, the remaining studies into four categories, and examines which experimental designs are most appropriate to measure reproductive costs. By this process he eliminates much of the literature as being unable to assess reproductive costs and is left with a small core of papers, which he proposes, properly test the theorem of the cost of reproduction.

The four categories are:

a) studies which measure the phenotypic correlations of present reproduction with future reproduction and/or survival;

b) studies which involve some manipulation of the organism's life-history and/or environment to affect change in its reproductive schedule;

c) studies which measure genetic correlations; and

d) studies which examine correlated responses to selection on some aspect of the life-history.

I have already dismissed the value of phenotypic correlations as measures of the costs of reproduction, because they can have no evolutionary consequences; they may also be misleading as they do not necessarily reflect underlying genetic correlations. Rose and Charlesworth (1981a) and Roach (1986) have found that the environmental correlations between life-history traits can often obscure negative genetic correlations, so that observed correlations will be positive. This confounding of environmental and genetic effects is even

more profound when comparing populations from different habitats or microhabitats, as do many phenotypic studies. Comparisons between populations run into the problems of comparing populations which may differ in their "fitness" or adaptation to the environment. The fitter population may well produce more offspring and live longer than the less fit population, but genetic correlations between reproduction and longevity may be negative within each population. Furthermore, a phenotypic correlation between two traits may be positive because both traits are positively genetically correlated with a third variable (Partridge and Harvey 1985).

Experimental manipulations of life-histories also do not measure genetic correlations but the phenotypic plasticity of the life-history (Reznick 1985). It seems likely that the pattern of energy allocation may be genetically set for a population of a species between soma and reproduction (Reznick 1982,1983; Springer and Boggs 1986). One is thus only witnessing the ability of a population to adjust phenotypically to changed environmental circumstances.

These studies also tend to compare non-reproducing with reproducing individuals, and thus, on one hand may overestimate costs, or underestimate them if the organism lacks the ability to respond to the manipulation (e.g. Reznick 1983). Also, manipulations of the environment are clumsy because "the causality underlying the correlation is as attributable to the controlled aspects of the environment as it is to a functional relationship between the life history variables" (Reznick 1985). Reznick (1985) believes that these types of studies have value insofar as they demonstrate how costs might be manifested in real organisms, and that these costs may be minimised through timing or cuing on resource availability. Environmental manipulations may identify possible ecological causes of reproductive cost (Bell 1986).

The examination of genetic correlations and responses to selection of populations are the most useful methods to evaluate the costs of reproduction. Both can establish if reproductive costs have a genetic basis (Reznick 1985), though they are subject to some limitations. Most studies investigating correlations between life-history traits only examine the population in one environment. This is usually because running a quantitative genetic analysis in a number of environments involves such large numbers that it is difficult to manage.

Genetic correlations may depend on the environment and genotype x environment interactions will affect the genetic correlations (Clark 1987a; Falconer 1981). "Novel" environments for the experimental organism are particularly likely to induce change in its

correlation matrix (Service and Rose 1985). Thus care should be taken that laboratory conditions approximate to the natural "realistic" conditions of the organism in evaluations of reproductive cost. However, I expect the genetic relationship between early fecundity and future fecundity and/or survival should always be negative in "realistic" environments if reproductive costs are fundamental constraints. If they are not fundamental, then testing a population in only one environment could be misleading.

McRae (1988) using two geographically distinct natural populations of *Tribolium castaneum* found that heritabilities and genetic correlations did not substantially differ for each population between two temperatures 33°C and 37°C. The magnitude of correlations between average daily fecundity and longevity sometimes altered but not their sign.

Genetic correlations also are highly dependent on gene frequency, and Reznick (1985) suggests that measurements on populations where gene frequencies are changing can yield highly variable results. This criticism though is only relevant to the accurate measurement of the magnitude of the correlations. It has little bearing on the matter of establishing the existence of reproductive costs, that is negative genetic correlations between early fecundity and late fecundity and/or survival.

Charlesworth (1984) has suggested that artificial selection experiments do not provide enough evidence to prove that there are negative genetic correlations between life-history components, as newly arising mutations may be responsible for the apparent correlations. However, the rate of reduction in fecundity in certain experiments (Rose and Charlesworth 1981b) has been so large that this explanation seems unlikely. Selection experiments do have the advantage of indicating how reproductive costs are expressed in the phenotype (Bell 1986).

Both types of studies tend to identify only the presence or absence of costs, not the nature of the trade-offs which is important when predicting the direction of life-history evolution (Bell 1984a; Reznick 1985).

Any work carried out under laboratory conditions can suffer from incomplete assessment of the costs of reproduction because it does not consider the costs that can be incurred through interactions between an individual and external factors in its environment (Reznick 1985). Such interactions may affect an individual's competitive ability, its susceptibility to disease or parasites, or susceptibility to predation. Most quantitative genetics experiments and selection experiments are necessarily set in the laboratory and thus can be criticised

for this reason. Koufopanou and Bell (1984) have attempted to assess the impact of predation on barren and pregnant female *Daphnia pulex* in a laboratory situation, in order to circumvent this problem.

In summary, Reznick (1985) concludes that in all the literature preceeding 1983 there are only ten papers that properly assess the costs of reproduction, and all of these support the presence of such costs. However, I would qualify his assessment as, on examination, most of the papers are by no means conclusive.

1.2.1.3 Empirical Evidence

The ten papers that Reznick (1985) cites are Morris (1963); Solbrig and Simpson (1974); Mertz (1975); Wallinga and Bakker (1978); Law (1979); Taylor and Condra (1980); Rose and Charlesworth (1981a,b); Hegmann and Dingle (1982); and Reznick (1983).

Morris (1963) had selected for high early egg production in White Leghorn hens for a number of years, and found indications that the genetic correlation between early and late reproduction changed from positive to negative during selection but could not statistically confirm the finding. As mentioned before, such a change in correlation is not unexpected during artificial selection (Falconer 1981; Murphy, Giesel and Manlove 1983; Sheridan and Barker 1974), and it certainly does not confirm the hypothesis that trade-offs between early and late reproduction mould life-histories..

Solbrig and Simpson (1974) can only be regarded as indicating a possible cost to reproduction. Whilst one strain (A) of the dandelion *Taraxacum officinale* set more seed than another strain (D), D outcompeted A in growth competition experiments. There is no information on age-specific aspects of reproduction and survival (Charlesworth 1980), thus it is impossible to link causally the greater vegetative growth with less reproductive output.

Mertz's (1975) results are more amenable to interpretation. Selection for early fecundity in a laboratory strain of *Tribolium castaneum* did enhance early fecundity and decrease late fecundity. However, this observation was not statistically confirmed, and there was no correlation between the fecundity response and the mortality response. The decrease in late life egg production may have been due to inbreeding, as suggested by the fact that lifetime egg production was greatest in lines not intensely selected.

Wallinga and Bakker (1978) appear to confirm that high early reproduction can be at the expense of late reproduction. The decrease in numbers of young in late litters of mice selected for large litter size, appears to be due to the accumulation of damage to the uterus

resulting from the early large litter sizes. If the female mice are allowed an interval between litters i.e. not mated until the previous litter is weaned, then there is no such observed decrease in litter size. Behavioural mechanisms then, which lead a female to reject a male until she has weaned her previous litter, could bypass this cost. On the other hand, natural selection could select for intermediate litter size, if the benefit of producing more young early was outweighed by the disadvantages of smaller late litters. Either way it is possible that this physiological limitation on reproduction provides a mechanism by which natural selection could mould age-specific reproductive strategy.

Wallinga and Bakker's (1978) results are further proof that the magnitude of a cost of reproduction can be diminished by temporal adjustment.

Law (1979) found various trade-offs between early reproduction and late reproduction in *Poa annua,* but no relationship between reproduction and mortality risk between seasons (years), though within a season there was an increased mortality risk correlated with high early reproduction. Perhaps, in this last instance, we are once more observing the importance of timing on the appearance of costs. Yet Law's (1979) results may not be proof of the existence of reproductive costs, in that his experimental methods have come in for strong criticism (Bell 1984a). In particular, any interaction between plant biotype and varying environmental conditions between years has been ignored.

Taylor and Condra's (1980) results seem to actually provide evidence against the existence of reproductive costs. The life-history traits of two populations of *Drosophila pseudoobscura* maintained under uncrowded and crowded conditions respectively were compared. The crowded population had better preadult viability and adult longevity. It also laid more eggs after an initial peak was reached, though no differences were evident between the two populations prior to this peak. There seems to be no trade-offs here, exactly the opposite, and I can only assume that Reznick (1985) interpreted the faster developmental rate of the uncrowded population, which allowed it to achieve oviposition earlier, as some sort of reproductive profit. However, there are no signs of the classic costs of reproduction. Comparing two populations is fraught with problems as explained before, when trying to establish the existence of reproductive costs.

Possibly the most quoted studies of support for the costs of reproduction are Rose and Charlesworth (1981a,b). Rose and Charlesworth (1981a) using a sib-analysis of an outbred laboratory population of *Drosophila melanogaster,* found negative genetic correlations between early fecundity and life-span, as well as between mean egg lay rate and life-span.

On close scrutiny their results do not completely warrant the confidence placed in them. Firstly, Rose and Charlesworth (1981a) did not include standard errors for their sib analysis, though they admitted the sampling variances were evidently large, so it is impossible to verify if the negative genetic correlations between early fecundity and life-span were statistically significant. They also did not get a consistently positive or negative correlation between longevity and late fecundity (Bell 1984a). However, if environmental effects are stronger later in life, then correlations are hard to obtain. The absence of a correlation could be taken as a sign of trade-off (reduced genetic control).

An artificial selection experiment for early fecundity (Rose and Charlesworth 1981b) obtained an increase in early fecundity but no corresponding decrease in late life parameters. Artificial selection for late fecundity did not improve late fecundity but did increase longevity and depress early egg lay. Yet, natural selection for late age fitness components increased late fecundity, female longevity and the duration of female reproduction, whilst also decreasing early fecundity. Thus Rose and Charlesworth's (1981b) results are somewhat conflicting as far as trade-offs between life-history parameters are concerned. These last experiments were not replicated and so cannot be used to provide quantitative estimates of genetic parameters (Falconer 1977).

Hegmann and Dingle (1982) examined the additive genetic variance-covariance structure influencing a set of life-history variables in the milkweed bug, *Oncopeltus fasciatus.* Amongst other variables, the clutch size of the first three clutches produced were measured, as was the interclutch interval. The only evidence of a reproductive cost was a negative genetic correlation between clutch size and interclutch interval. However, the standard errors for the genetic correlations for each of the first two clutches with interclutch interval were so huge that these correlations are meaningless. Thus the only proof of a reproductive cost is a negative genetic correlation between the size of the third clutch and the interclutch interval.

Hegmann and Dingle (1982) combined data for their analyses from three samples derived from the wild over a period of two years. They have made the assumption that the sampled population has undergone little genetic change in the field, but with little support for this assumption. It is questionable whether they are examining the same population, and this factor may explain why their results are inconsistent for the clutch-interclutch correlations.

Reznick (1983) does establish that there is a genetic basis to the partitioning of energy in the guppy *(Poecilia reticulata),* and that within an age-class increased somatic investment

(growth) is at the expense of reproductive investment (reproduction). He then extrapolates that, since fecundity is directly proportional to size in guppies, there is a trade-off between current and future reproduction. However, there is no direct proof of a cost of reproduction so this study also remains somewhat inconclusive.

In summary, only Wallinga and Bakker (1978) seem to provide sound evidence for the existence of a cost to reproduction. Unfortunately, they used a long-term laboratory population, and obviously their work is not enough to substantiate or generalise reproductive cost as an important evolutionary force in all life-histories.

Since 1982, more papers have been published which are relevant to this area, and I have tried to assess their value, as well as that of some papers published before 1982 which were not evaluated by Reznick (1985). These papers are listed in Table 1.1. I have used the same system of classification as Reznick (1985), and will restrict detailed review of papers to those which determine the genetic correlations between early and late life-history traits, or are interesting for their practical demonstration of costs.

Koufopanou and Bell's (1984) results provide conflicting evidence, and they are classified into both categories. Their laboratory study tried to demonstrate the effects of predation on pregnant guppies, and thus to illustrate one type of reproductive cost not usually measurable in the laboratory. One major problem with this type of experiment is that it does not allow for the possible modification of behaviour in the wild by pregnant guppies which may mediate such a cost. Koufopanou and Bell (1984) attempted to show increasing vulnerability to predation with each egg added to a female's brood. Two such experiments related survival rate to body size, and only one found a negative correlation between survival and fecundity. Of course, this is not strong evidence for a reproductive cost as such, and Koufopanou and Bell (1984) conclude that the effect of size on life-history parameters may be stronger than the antagonistic effects among life-history parameters.

Templeton (1982,1983), Templeton and Rankin (1978), and Templeton and Johnston (1978) have examined the abnormal abdomen (aa) syndrome in *Drosophila mercatorum*. The appearance of abnormal abdomen is controlled by a single X-linked "locus" with two alleles (aa and +). The syndrome is temperature sensitive and, apart from disrupting the development of the abdomen, affects adults, even heterozygotes, by increasing the rate of ovarian maturation and egg production and decreasing longevity (Templeton 1982,1983). This seems to provide evidence for a genetically based trade-off between reproduction and longevity. However, major genes with pleiotropic effects on reproduction and longevity are

Table 1.1: Experimental work on reproductive cost since 1982.

Class of Paper	Reproductive Costs	
	Present	**Absent**
Phenotypic		
	Aigaki and Ohba (1984)	Morris (1986)
	Ekman and Askenmo (1986)	Warner (1984)
	Kimura and Tsubaki (1986)	
	Maltby and Calow (1986)	
	Meagher and Antonovics (1982)	
	Millar and Zammuto (1983)	
	Zammuto (1987)	
Experimental manipulations	Collatz and Wilps (1985)	Collatz and Wilps (1983)
	Koufopanou and Bell (1984)	Koufopanou and Bell (1984)
	Maynard Smith (1958)	
	Nur (1988)	
Genetic correlations	Friedman and Johnson (1988)	
	Hiraizumi (1985)	Bell (1984a,b)
	McRae (1988)	Butlin and Day (1985)
	Service and Rose (1985)	Murphy, Giesel and Manlove
	Templeton (1982,1983)	(1983)
	Templeton and Johnston (1982)	
	Tucić, Cvetković and	
	Milanović (1988)	
Selection responses	Luckinbill, Arking, Clare,	Gilbert (1984a,c)
	Cirocco and Buck (1984)	Druger and Matzke (1977)
	Luckinbill and Clare (1985)	Stearns (1983a)
	Rose (1984a)	

unlikely to provide a general basis for trade-offs between these life-history traits. Abnormal abdomen does appear to be due to disruption of the developmental processes in late third instar larvae or prepupae (Templeton and Rankin 1978), and, since all life-history traits appear to be developmentally linked, perhaps it is to be expected that they would all be affected. Butlin and Day's (1985) results must also be treated with caution because they too are examining the action of a "major gene equivalent" with pleiotropic effects on life-history characters.

Friedman and Johnson (1988) have found a recessive mutant allele in *Caenorhabditis elegans* that lengthens life-span, reduces reproductive fitness but affects no other traits. The wild allele is favoured by natural selection because it increases reproductive fitness fourfold and only decreases the length of postreproductive life which has no evolutionary consequences. The action of this mutant allele does demonstrate that a genetically based trade-off between longevity and reproduction can and does exist in this species.

Service and Rose (1985) found a significant negative genetic correlation between early life fecundity and starvation resistance, which is correlated with normal life-span (Service, Hutchinson, MacKinley and Rose 1985) in *Drosophila.* Using starvation resistance as an index of longevity means that the observed negative genetic correlation is only suggestive of a relationship between longevity and early fecundity. Their results are also somewhat qualified as they used the same population as Rose and Charlesworth (1981a,b), thus they have only confirmed that negative genetic correlations between early and late life-history traits may exist in this one particular population. Rose (1984a) selected for late fecundity in that same population, and also confirmed the existence of the trade-offs found by Rose and Charlesworth (1981b).

Luckinbill *et al.* (1984) and Luckinbill and Clare (1985) selected for late and early fecundity in a different laboratory strain of *Drosophila melanogaster,* but like Rose and Charlesworth (1981b), found that while selection for late fecundity did produce early life trade-offs, selection for early fecundity did not increase it at the expense of late life traits.

Hiraizumi (1985), using isofemale and chromosome replacement lines of *Drosophila melanogaster,* found that lines with the highest early oviposition ceased laying eggs at a younger age and died relatively earlier. This is strong evidence for a survival cost but, as Clark (1987a) points out, such lines are genetically artificial and "the structure of expressed variation and covariation may be limited to the artificial conditions".

If we are really interested in establishing the existence and importance of reproductive

costs in the moulding of life-histories, then we must establish that these costs exist in natural populations. Bell (1984a,b), Gilbert (1984a,c), McRae (1988), Murphy *et al.* (1983), Stearns (1983a) and Tucić *et al.* (1988) have all examined various natural populations for evidence of such costs.

Murphy *et al.*'s (1983) results are only suggestive that there is no trade-off between early reproduction and longevity because of unusual indices of reproduction used. Furthermore, no measure was taken of the relationship between early and late reproduction.

Stearns (1983a) also found no significant association between fecundity and longevity, but his results also are suspect, as he did not examine the correlation between early and late reproduction, and for his measurement of fecundity only used one litter of each female with an age range between 7 and 15 months.

Bell (1984a,b) took measures of both fecundity and survival costs in the rotifer, *Platyias patulus,* and five other asexual freshwater invertebrates, all populations recently derived from the wild. He concluded that there was no evidence of reproductive cost within species.

Reznick, Perry and Travis (1986) have criticised Bell's (1984a,b) statistical analyses and genetic interpretation. Bell (1986) has reanalysed his fecundity data from (1984b) and still found that genetic correlations were predominantly positive. Unfortunately he did not reestimate the genetic correlations between survival and fecundity.

Druger and Matzke (1977) also found no reproductive trade-offs in a laboratory population of *Drosophila pseudoobscura.* They compared two populations which had been maintained under different environmental conditions in the laboratory for fifteen years. I have two criticisms of this study. Firstly, the two base populations were different though related, so comparing their selection responses is probably not strictly valid. Also, although there was no correlation between egg lay and longevity and no difference in total egg lay between the two populations, the shape of the oviposition curves of the two populations did differ. This difference was not examined and it may have revealed a trade-off between early and late reproduction and/or longevity.

Gilbert (1984a) examined a wild population of cabbage white butterfly, *Pieris rapae,* subjected to natural selection pressures in the field, after establishing a genetic basis for individual differences in fecundity. He found larger cabbage white butterflies have an advantage in fecundity throughout their lifetime, which is not counteracted by reduced survival (Gilbert 1984a). However, Gilbert (1984c) found that artificial selection for body weight quickly leads to a selection limit which is produced by reduced fecundity, and that it is the

genotype of the butterfly, not its actual size, that is responsible for the loss in fecundity. Of course such a reduction in fecundity also could be the result of inbreeding, but measures were taken to avoid inbreeding and butterflies selected for small body size suffered no similar reduction in fecundity. Obviously, Gilbert's (1984c) results do not confirm the existence of classic costs of reproduction as they concern the trade-off between size and reproduction.

McRae (1988) has provided the most substantial support for the theorem of reproductive cost. Negative genetic correlations between early reproductive traits and adult life-span were found in two populations of *Tribolium castaneum* recently derived from the wild. Both populations were maintained at two temperatures (33°C and 37°C). Correlations between early and late life fecundity indices were consistently positive within both populations at both temperatures. Thus reproductive cost manifested itself as a survival cost, not a fecundity cost in these two populations.

Tucić *et al.* (1988) have found both survival and fecundity costs in *D. melanogaster* females sampled from a natural population. There were no significant genetic correlations between male traits, though male virility was negatively correlated with female late fecundity and longevity.

On the basis of all the above papers, I would conclude that the universal applicability of the cost of reproduction has not been established for natural populations. It is obvious that more work is required in this area before we can accept reproductive cost as the basis of life-history evolution.

1.3 The Evolution of Senescence

1.3.1 Introduction

The ultimate constraint on the fitness of an individual is death. Death may be caused by external factors, but it is innate in almost all living organisms, for even in optimal environments organisms age and die. The rapid increase in the force of mortality after a period of time which is characteristic for each species is termed senescence (Kirkwood and Holliday 1979).

Senescence (ageing) commences at an age after the onset of reproductive maturity (Rose 1984a) and not only signals the decline of age-specific survival probabilities but also the age-specific fecundity of individuals (Charlesworth 1980).

Senescence is due to the failure of many different physiological functions which affect

Table 1.2: Evolutionary theories of senescence.

Evolutionary theories of senescence	
Indirect Selection	**Direct Selection**
Developmental Hypothesis	Running-out-of-program
Antagonistic Pleiotropy	Group Selection Hypothesis
Unitary Hypothesis	
Deleterious Mutation Accumulation	

viability, and in turn decrease ability to cope with the environment (Comfort 1979; Lamb 1977). These two factors, internal and external, are responsible for the increasing susceptibility to death with age. Death is the culmination of senescence and its timing is "determined by the innate rate of physiological decline ... and the severity of the extrinsic challenges destabilising its homeostatic balance" (Sohal 1985).

Obviously senescence is a deleterious trait at the level of the individual but it is an almost universal component of life-histories. The unanswered question is why has senescence not been eliminated by natural selection?

There is an abundance of theories (Table 1.2), not always mutually exclusive, which attempt to answer this question. Theories range from the purely stochastic, that is ageing is a result of random damaging events which may be intrinsic or extrinsic in origin, to the programmatic where ageing is percieved to be under the direct control of specific genes (Sohal 1985). Within the spectrum of genetic theories, senescence is variously viewed as a non-adaptive trait, which is the result of indirect selection, or as a trait whose evolution and/or postponement is a direct consequence of natural selection.

This section reviews the major evolutionary theories concerning senescence. Each theory is presented separately, but it should not be inferred that they are mutually exclusive. The evolution of senescence may be due to a number of factors, and the important factors may differ between species.

1.3.2 Stochastic Theories

The unifying assumption of stochastic theories is that there is no underlying genetic basis to senescence. Senescence is due to damage incurred by the organism during the course of life. Thus senescence is the result of random damaging events which may be extrinsic or

intrinsic in origin (Sohal 1985).

The oldest and most obvious theory is that ageing is due to cellular "wear and tear" (Weismann 1891). This theory has been criticised for being far too simplistic in comparing senescence of living organisms, which have regenerative properties, to the breakdown of machines, which have none (Williams 1957).

However, many others have conjectured that senescence is due to the accumulation of randomly occurring damaging changes within macromolecules. Various mechanisms postulated are somatic mutation (Curtis 1967); cross-linkages within proteins and DNA (Bjorksten 1974); free-radical damage (Gordon 1974); and a progressive decrease in the accuracy of protein synthesis, i.e. the "error catastrophe" hypothesis (Orgel 1963).

The problem with all these mechanisms is that they do not explain the existence of differences between species in rates and patterns of ageing and length of life-span (Sohal 1985; Sonneborn 1978). The existence of such differences strongly suggest, if not prove, that ageing has to have a genetic component. There is much experimental evidence also supporting this hypothesis (e.g. Bell 1984a,b; Bourgois and Lints 1982; Clare and Luckinbill 1985; Clarke and Maynard Smith 1955; Johnson and Wood 1982; Luckinbill 1985; Luckinbill and Clare 1985,1986; Luckinbill, Graves, Reed and Koetsawang 1988; Roberts 1961; Rose 1984a; Rose and Charlesworth 1981a,b; Service 1987; Service, Hutchinson and Rose 1988; Wattiaux 1968b). In particular, there is direct evidence that the control of longevity is polygenic (Luckinbill *et al.* 1988; Service 1987).

It is likely that all the postulated mechanisms mentioned above, are just that: mechanisms of senescence, and are the end-product or products of genes "which are themselves the subject of genetic and evolutionary theories" (Luckinbill *et al.* 1984).

1.3.3 Genetic Theories

1.3.3.1 Developmental Hypothesis

The developmental hypothesis of ageing considers senescence and death as the programmed ultimate stage of differentiation and development (Lints 1963; Muller 1963). There are no specific genes controlling longevity (Lints, Stoll, Gruwez and Lints 1979) and longevity is not subject to the direct action of natural selection (Lints 1978a). Rather, it is epigenetically controlled "i.e. a trait whose expression is linked to the regulation of gene function, of differentiation, or of the topographic distribution and function of proteins" (Lints 1988).

Supposedly, environmental influences during development affect the expression of genes controlling developmental processes and these changes in expression affect the duration of life-span (Soliman and Lints).

There are two lines of experimental evidence which have supported this hypothesis.

Firstly, there are many examples of a close link between development/growth rate and life-span. Negative correlations between growth rate or duration of development and longevity have been found in many species (e.g. Eklund and Bradford 1977; Goodrick 1978; Lints and Lints 1971b; Marinković and Tucić 1982; Ross, Lustbader and Bras 1976; Smit, Rizova, Janković, Mihailović, Jovanović and Tucić 1981; Soliman and Lints 1982), whilst variations in growth rate have been shown to be correlated with variations in life-span within species (e.g. Lints and Lints 1971; Ross *et al.* 1976). However, not all experimental work has found a negative relationship between growth rate or duration of development and longevity (Bourgois and Lints 1982; Cohet 1975; Cohet and David 1976; Economos and Lints 1984a,b,c,1985; Mayer and Baker 1984). Tucić, Cvetković and Milanović (1988) in particular have found a positive genetic correlation between duration of development and longevity in females sampled from a natural population of *Drosophila melanogaster.* Economos and Lints (1984a,b,c,1985) experimentally manipulated the growth rate and life-span of *Drosophila* by varying the amount of yeast provided in media and concluded that there was no relationship between duration of development and life-span. Any correlation between the two could not be taken as evidence of a direct causal relationship (Economos and Lints 1985). Economos and Lints (1986a,b) investigated the effect of developmental temperatures on the development and longevity of *D. melanogaster.* They found no direct relationship between growth rate and life-span, but rather an optimal range of temperature, with life-span decreasing sharply on both sides of that range due to disruption of normal developmental processes. Other workers have confirmed these findings for other species (Lints 1988a). Obviously, the developmental hypothesis is not generally supported by this body of work.

The second line of experimental evidence was the lack of response to indirect and/or direct selection for longer life-span in *D. melanogaster* (Lints and Hoste 1974,1977; Lints *et al.* 1979). Bourgois and Lints (1982) found genetic divergences between subpopulations of *D. melanogaster,* maintained at three different temperature regimes, for duration of development, size, growth rate and life-span. They believed that the phenotypic expression of life-span was not linked to acclimation temperature and thus that longevity was not "solely"

subject to the direct action of natural selection. In the light of the results of Economos and Lints (1986a,b), Bourgois and Lints' (1982) findings are probably attributable to the disruption of developmental processes, or, perhaps merely the result of genetic drift.

Response in longevity to selection for late reproduction has been shown by Luckinbill *et al.* (1984), Rose (1984a) and Rose and Charlesworth (1980, 1981b), so that the lack of response to selection in the studies of Lints and others was probably an artifact, due to larval density. Lints and Hoste (1974,1977) and Lints *et al.* (1979) used low larval density during their selection experiments. However, Clare and Luckinbill (1985) and Luckinbill and Clare (1985,1986) have shown that when larval density is held low, selection fails in *D. melanogaster* and life-span fluctuates; but when it is uncontrolled, selection for late reproduction dramatically increases longevity. Arking and Clare (1986), Clare and Luckinbill (1985) and Luckinbill and Clare (1985) have proposed that in non-stressful environments, developmental buffering systems suppress the expression of genetic variation. In stressful environments (i.e. uncontrolled density) the developmental buffering fails, and the genetic variation is exposed to selection.

Clare and Luckinbill (1985) have shown that genes for short life are dominant at low densities, but display additive inheritance at high densities. Gilbert (1986) found a similar maximisation of expression of genetic variation for developmental rate in a butterfly, *Pieris rapae,* at stressfully low temperatures. Other studies also have shown that variation in gene expression can occur as a function of such gene-environment interaction (Derr 1980; Murphy, Giesel and Manlove 1983; Parsons 1975,1977,1978,1982,1987; Robertson 1961,1963,1964,1966). Thus longevity does seem to be an adaptable feature of life-history and directly modifiable by natural selection. However, a link between development and longevity cannot be ignored. There is definitely some form of modification of longevity by environmental conditioning during development, at least in *D. melanogaster* (Economos and Lints 1984a,b,c,1985; Luckinbill and Clare 1986; Sondhi 1968), and the extent of modification is determined genetically (Marks 1982). Yet, senescence in *Drosophila melanogaster* is not a mere continuation of developmental processes. It needs to be shown in other species apart from *D. melanogaster,* that senescence is not epigenetically determined before the present form of the developmental hypothesis is abandoned.

Correlates of the developmental hypothesis of ageing are:

1) duration of development and/or growth rate are correlated with longevity;

2) modification of development by selection should alter longevity in the direction of the sign of the correlation;

3) modification of other life-history traits by selection, such as early and late fecundity, should have no effect on longevity, providing developmental rate is unaffected; and

4) longevity does not respond to direct selection.

1.3.3.2 Antagonistic Pleiotropy

According to this hypothesis, ageing and senescence are the result of the action of genes which maximise early fitness (reproduction and viability), but have deleterious effects later in the life-span (Medawar 1952; Williams 1957). Selection favouring such genes is due to the decreasing force of natural selection with advancing age (Charlesworth 1980; Haldane 1941; Hamilton 1966; Medawar 1952; Williams 1957). Even in the absence of ageing the pressures of natural selection weaken with age because "accidents", outside the action of senescence, cause there to be less surviving individuals in each successive portion of the life-span and thus decrease the probability of reproduction with increasing age.

The force of natural selection at any given age is a function of the fraction of the average total lifetime expectation of reproduction which occurs after that age. Concomitantly natural selection acts most forcefully on those portions of the life-span where the value of future expected reproduction is at its greatest i.e. at reproductive maturity. Thus reproduction should be more highly variable among older individuals if late fecundity is less important than early fecundity (Bell 1980). Theoretical models have shown that a gene which causes a small increase in survival or fecundity early in life, may be selected for, even if it has a pleiotropic effect decreasing the probability of survival later in the reproductive period (Charlesworth 1980; Hamilton 1966).

Senescence is a compromise between two forces:

1) direct selection against senescence in the early stages of reproductive life because it is an unfavourable trait; and

2) indirect selection for senescence due to selection favouring alleles with pleiotropic late life deleterious effects (Williams 1957).

The age at which harmful effects appear would be determined by the distribution of

adult mortality and the patterns of reproduction. Low adult death rates and an increasing fecundity after maturity should delay senescence, whilst high adult death rates and decreasing fecundity once past reproductive maturity should hasten senescence. The antagonistic pleiotropy hypothesis is only applicable to the evolution of life-history differences among individuals in the same breeding population confronted by the same environmental constraints (Schnebel and Grossfield 1988).

The hypothesis has been criticised for assuming that organisms were originally immortal (Cutler 1978; King 1982; Sacher 1978a), but whether senescence was inherent or evolved is still a subject of discussion (e.g. Cutler 1976; Nanney 1974; Sacher 1978b; Sonneborn 1978). However, Williams (1957) only postulated immortality as one extreme, in order to prove that even if senescence did not exist, it could evolve by the mechanism of antagonistic pleiotropy. If senescence was inherent, then antagonistic pleiotropy would ensure it was not eliminated by natural selection.

Opponents of the theorem also have suggested that it relies on genes being able to tell the time, i.e. early versus late life, but genes cannot do this unless "ageing" processes are already existent (Calow 1978; Kirkwood 1977; Kirkwood and Holliday 1979; Sacher 1978a). This criticism is only valid if senescence is not an innate feature of life. Even if senescence did evolve there is not a necessary circularity in Williams' (1957) hypothesis. Non-senescent life-history changes, such as growth in size (Charlesworth 1980), random events (Nanney 1974), or genes whose products decay over an interval of time, extending into the reproductive period of life, could all be used by primitive organisms to measure biological time. There are also genes whose function may not change with age, but whose effects may change from beneficial to deleterious. For example, Cutler (1978) suggested that genes which code for calcium uptake for bones in a young animal may be responsible for calcification of the connective tissues in arteries as it grows past maturity.

Kirkwood and Holliday (1986) have proposed that the "pleiotropic" genes govern the way resources are allocated among the various tasks an organism must perform and are independent of timing. Briefly, their "disposable soma" mechanism proposes that higher organisms adopt energy saving strategies of reduced accuracy in proofreading of DNA in somatic cells to accelerate development and reproduction, but the consequence will be eventual deterioration and death (Kirkwood and Holliday 1979).

Evidence supporting the antagonistic pleiotropy theory derives from:

1) there are pleiotropic genes which have different and opposite effects on fitness at different ages, or, more accurately in different somatic environments (Caspari 1950,1954; Gonzales 1923; Simmons, Preston and Engels 1980); and

2) empirical evidence has shown that there are negative genetic correlations between early and late life indices in populations of *D. melanogaster* and *T. castaneum.*

One group working with the same *D. melanogaster* laboratory population has found negative genetic correlations between early fecundity and life-span (Rose and Charlesworth 1981a) and by selecting for late fecundity have increased it and longevity at the expense of early fecundity (Rose 1984a; Rose and Charlesworth 1981b). Early fecundity has also been shown to be negatively correlated with positive correlates of life-span (Service *et al.* 1985; Service and Rose 1985). Luckinbill *et al.* (1984) and Luckinbill and Clare (1985) using a different hybrid laboratory strain of *D. melanogaster* obtained similar results to Rose and Charlesworth (1981b) and Rose (1984a). Tucić *et al.* (1988) found significant negative genetic correlations between early fecundity and late fecundity, and early fecundity and longevity in *D. melanogaster* sampled from a natural population.

Selection for early life fecundity in *T. castaneum* depressed longevity in two different laboratory populations (Mertz 1975; Sokal 1970). McRae (1988) found negative genetic correlations between early reproductive traits and adult life-span in two populations of *T. castaneum* recently derived from the wild, maintained at two temperatures (33°C and 37°C).

However, there has been criticism of the empirical work which purports to support the antagonistic pleiotropy theorem.

Firstly, the experimental design of much of the work has been regarded as inadequate. Selection for increased late fecundity has supposedly shown a positive genetic correlation between longevity and late fecundity, but selecting for increased late fecundity is contingent on survivorship (Clark 1987a). Thus there is inadvertent indirect selection for longevity as well as late reproduction. Increasing longevity by selecting for increased late fecundity cannot be used as proof.

Lansing effects also may confound the results of experiments which investigate the evolutionary theories of senescence by selection for early and/or late fecundity (Lints and Hoste 1977). Lansing effects are cumulative, transmissable effects due to repeated reproduction

at the same time of the life cycle through successive parental generations. If Lansing effects do exist, which is still debated (Goodnight 1988; Lints 1988b), they are spontaneously reversible, so perhaps would not affect long-term selection experiments.

Further, according to Clark (1987a), the experimental design of both genetic variance and covariance analyses and selection experiments ignores the fact that "genetic correlations may depend on the environment, and genotype x environment interactions will affect the genetic correlations". However, as discussed previously (section 1.2.2.2), genetic correlations between important life-history characters are expected to be constant in sign, if not in magnitude, if measured in conditions that are realistic for the experimental organism. If the antagonistic pleiotropy theorem is correct, genetic correlations between early fecundity and late fecundity or survival should always be negative in conditions that approximate to those in nature for the organism.

Researchers need to be careful that negative genetic correlations between early and late life indices are the result of unavoidable genetic and physiological constraints (Istock 1983). Genetic correlations between simultaneously selected traits will become negative with time (Falconer 1981; Sheridan and Barker 1974) and a negative genetic correlation between early and late reproduction may be the result of natural selection maximising early and late reproduction. Testing a range of populations over a range of realistic environments should reveal whether a negative genetic correlation is universal.

It may be easier to disprove the antagonistic pleiotropy theorem than to prove it.

Much of the empirical work testing the theorem has not been conclusive and sometimes has produced results which seem to conflict with it. For example, selection for early fecundity did not depress longevity or late fecundity (Luckinbill *et al.* 1984; Luckinbill and Clare 1985; Rose and Charlesworth 1981b). Since early fecundity responded to selection, it is unlikely that the lack of indirect responses was due to a lack of heritable variability for early fecundity, as suggested by Rose and Charlesworth (1981b). Mertz (1975) found that the depression in longevity of most lines selected for high early fecundity was heterogeneous and not correlated with the mortality response. He concluded the reduction in longevity was brought about by the absence of selection for deferred senescence. McRae (1988) found positive genetic correlations between early and late life fecundity indices in two populations of *Tribolium* at two temperatures. Such genetic correlations in this long-lived species (Dawson 1977) are unlikely if the theorem is correct, as late fecundity should be detrimentally affected by alleles that improve early fecundity. The recessive mutation found by Friedman

and Johnson (1988) in *Caenorhabditis elegans,* which lengthens life-span at the expense of reproductive fitness, is compatible with the antagonistic pleiotropy theorem of senescence. Clearly though this does not prove that senescence is due solely to the action of such genes.

Rose *et al.* (1987) and Service, Hutchinson and Rose (1988) have concluded, on investigating subsidiary characters contributing to longevity in the population of *Drosophila* used by Rose in his previous work, that antagonistic pleiotropy cannot be the sole mechanism for the evolution of senescence. It may act in combination with other forces, or it may be unimportant. On the other hand, its importance may vary from species to species. Clearly more empirical work needs to be done in different species.

As a final caveat to the antagonistic pleiotropy theory of senescence, there is a body of work from the laboratory of J.T. Giesel which has found positive genetic correlations between early fecundity and longevity in populations of *Drosophila.* These papers will be reviewed in the following section, as they form the basis of another theory about the evolution of senescence.

Testable predictions of the antagonistic pleiotropy hypothesis are:

1) negative genetic correlation between early fecundity and longevity; and

2) negative genetic correlation between early fecundity and late fecundity.

1.3.3.3 Unitary Hypothesis

Rose (1983a) was the first to categorise this theory. It was presented as a general theory of life-history evolution, which of course must cover the evolution of senescence. As a general theory, it proposes that all life-history characters are inextricably bound by their positive correlations with fitness (Giesel and Zettler 1980). That is, genes which code for one life-history character, codetermine all others e.g. "reproduction and the survival characteristic are generally inseparable" (Giesel 1979). According to Dobzhansky (1968), increased length of life-span is the indirect response to selection for improvement in other life-history traits which improve overall vigour.

In support of this hypothesis some studies have shown positive genetic correlations among life-history traits (Giesel 1979, 1986; Giesel *et al.* 1982a,b; Giesel and Manlove 1983; Giesel and Zettler 1980; Murphy *et al.* 1983). One would predict that natural selection would favour genes with beneficial pleiotropic effects strongly and genetic variation in life-history traits would quickly disappear. But this is not the case.

Giesel *et al.* (1982a,b) and Murphy *et al.* (1983) have hypothesised that genetic variation for fitness traits remains because of the interaction between genotype-environment interactions and fluctuating selection pressures in heterogeneous environments. Most natural populations experience temporally heterogeneous environments, so genetic correlations between fitness traits in a given environment should be positive, whilst correlations across environments between expressions of the same life-history trait should be negative (Baldwin and Dingle 1986; Via 1984).

However, if selection were consistent and unidirectional, as in a long-term laboratory population or natural population in a "constant" environment, negative genetic correlations between fitness traits would predominate. Alleles which had beneficial effects on traits within the specific environment, would be selected to fixation leaving only those with antagonistic effects on life-history traits to segregate (Falconer 1981). The Unitary hypothesis then is really a version of Wright's (1977) "universal pleiotropy" between life-history traits.

Concerning the evolution of senescence, the assumption is that senescence is an inherent attribute of multicellular organisms (Giesel 1979), as there is no way to explain the evolution of this deleterious trait within the bounds of this theorem.

Work supporting the Unitary hypothesis has originated from one laboratory (Giesel 1979,1986; Giesel *et al.* 1982a,b; Giesel and Manlove 1983; Giesel and Zettler 1980; Murphy *et al.* 1983). Studies show positive genetic correlations between early and late fecundity and longevity using *Drosophila* populations recently derived from the wild. However, the experimental design of these studies has been strongly criticised.

Service and Rose (1985) have suggested that the results are invalid because genetic correlations determined under "novel" conditions may be systematically biased toward positive values. Further, they suggest that because of this bias, populations recently derived from the wild are not suitable for experimentation. Service and Rose's (1985) paper does not justify this assertion. Firstly, the negative correlations between life-history traits in their laboratory-adapted population were only reduced in magnitude in the new environment. Secondly, the novel environment used was severely stressful. The high mortality in this stressful environment resulted in the comparison of the original population with a selected subpopulation. This selected subpopulation could be comprised of individuals who were more adapted to this environment, and whose genetic correlation matrices may not have truly reflected those of the entire population. Thirdly, Pashley (1988) has shown that novel

conditions can uncouple genetically correlated characters in fall armyworms so that genetic correlations change to insignificant, either becoming more positive or more negative dependent upon the traits measured. He found no systematic bias toward positive values. Finally, the results of placing a laboratory population into novel conditions, as Service and Rose (1985) did, may not be strictly applicable to populations recently derived from the wild. Wild populations, particularly of colonising species like *Drosophila* are more adapted to experiencing "novel" conditions than laboratory populations adapted to one set of constant environmental conditions. The effects on the genetic variance-covariance matrix may be quite different in a wild population or one recently derived from the wild.

Giesel (1979), Giesel and Zettler (1980) and Giesel, Murphy and Manlove (1982a,b) have been rightfully criticised because of their use of inbred lines. Positive genetic correlations among fitness components may be generated by inbreeding if rare deleterious alleles have been fixed (Mackay 1985; Rose and Charlesworth 1981a). Rose (1984b) also has demonstrated that there is no necessary relationship between the genetic covariation patterns of life-history characters found from inbred lines and the outbred populations from which they were derived.

Giesel *et al.* (1982a,b) used some outcrossed lines but were not able to separate dominance variation nor maternally-mediated environmental effects (Falconer 1981) from the estimates of heritability, because a full-sib design was used. Maternally-mediated environmental effects would tend to make all correlations more positive in value (Reznick 1985).

Finally, Giesel (1986) and Murphy *et al.* (1983), used such unusual and limited indices, such as instantaneous birth rate (IBR), that any conclusions about the relationship between fecundity and longevity were unwarranted. Peak fecundity was the only measure of early fecundity (which may or may not be a good guide), while there was no measure of late fecundity. It is difficult to compare the heritabilities and correlations with those in other papers which have used such characters as late and early fecundity, age at death and developmental rate (e.g. Giesel *et al.* 1982a,b; McRae 1988; Rose and Charlesworth 1981a). Since fecundity in the first five days was measured in order to derive IBR, it is difficult to understand why genetic correlations between it and age at death were not calculated. Further, the work of Giesel and his collaborators suffers from small observational numbers and, subsequently, degrees of freedom.

Results from other studies also undermine the validity of the Unitary hypothesis. Firstly, negative genetic correlations between fecundity and longevity have been found in laboratory

populations recently derived from the wild (McRae 1988; Tucić *et al.* 1988). This is not necessarily proof against the theorem but does raise questions. Secondly, environmental heterogeneity may not be responsible for maintaining genetic variation of fitness components. Not only have some workers found no increase in additive genetic variation with environmental variability (Dawson and Riddle 1983; Riddle, Dawson and Zirkle 1986), but laboratory populations maintained under constant laboratory conditions can exhibit high equilibrium levels of genetic variation in life-history characters (Rose 1983a; Rose *et al.* 1987). Furthermore, positive genetic correlations have been found across environments for important age-specific life-history traits (Groeters and Dingle 1987).

As Clark (1987a) points out, Giesel has yet to prove that negative genetic correlations between traits are the result of a stable environment.

If the Unitary hypothesis is correct then one would expect future experimental work to show:

1) insignificant to positive genetic correlations between early and late life-history traits in populations which have not been in the laboratory for many generations;

2) negative genetic correlations between expressions of the same age-specific life-history trait measured in different environments for such a population; and

3) genetic improvement in one life-history trait should affect all others, usually in a beneficial fashion.

1.3.3.4 Deleterious Mutation Accumulation

This hypothesis is based on the same assumption as the Antagonistic Pleiotropy theorem i.e. natural selection acts most forcefully on those portions of the life-span where the value of future expected reproduction is at its greatest; and declines with the decline in value of future reproduction (Charlesworth 1980; Hamilton 1966; Medawar 1952).

Senescence has evolved because of the virtual absence of selection against genes with deleterious effects in old age (Edney and Gill 1968; Emlen 1970; Medawar 1952). Deleterious mutations which have an early "age-of-onset" face strong selection pressure to eliminate them, whereas those whose effects occur at later stages of the reproductive period are allowed to accumulate (Charlesworth 1980).

A corollary of this theory is an expected increase with age in the additive genetic variance for fitness components, because selection-mutation equilibrium frequencies for deleterious

genes increase with age (Charlesworth 1980; Rose 1983a; Rose and Charlesworth 1981a; Rose et al. 1987). Thus a life-history character, such as reproduction, should show greater additive genetic variance in the older than in the younger animal. However, declining allelic effects (Rose and Charlesworth 1981a) or loss of alleles due to mortality (Clark 1987a) may respectively hide or prevent this increase in additive genetic variation.

Empirical evidence tends to be conflicting: Rose (1979) and Rose and Charlesworth (1980,1981a,b) found no increase in additive genetic variance for *Drosophila* age-specific fecundity, whereas Tucić *et al.* (1988) did. Liljedahl, Gavora, Fairfull and Gowe (1984) found for chickens an increase with age in the additive genetic variance for egg numbers. However, although the standard deviation increased with decreasing mean egg numbers, the data were not transformed, so their results must be treated with caution.

Another corollary of the theorem is that there should be little relationship between early and late life history characters in a population at linkage equilibrium, as late life characters are influenced by deleterious alleles that do not affect early life characters. This is particularly true of the late life trait, age at death, which should be solely determined by the action of these deleterious alleles.

The Mutation Accumulation hypothesis predicts:

1) little genetic correlation between early life history characters and longevity; and maybe

2) an increase in additive genetic variation of fecundity with age.

1.3.3.5 Running-out-of-Program

The premises of the Running-out-of-Program (RP) evolutionary theory of ageing are that, senescence is an inherent feature of organisms, there is natural selection for longer life and this is mediated by positive genetically controlled mechanisms (Cutler 1978,1980,1982; Sacher 1978a,1982).

Cutler (1978) proposed that senescent (biosenescent) and antisenescent (antibiosenescent) processes have evolved together. Life itself is an antibiosenescent process developed to preserve information, which continues to preserve itself. Protective and repair processes were incorporated into the original unicellular organisms to deal with external hazards, natural instability of the biological constituents of the living system and harmful intrinsic pleiotropic effects of metabolism and development (Cutler 1980). Cell division was one way multicellular organisms could maintain a type of immortality e.g. the sea anemone.

However, differentiation of the more complex multicellular organisms placed a limit on cell division as an antibiosenescent process. This led to the separation of organisms into mortal soma and immortal germplasm.

Antibiosenescent processes exist only to the extent necessary to ensure the maximum probability of survival of the genetic information of the organism. Ageing and timing of death are moulded by the reproductive pattern of a species. Cutler (1978) agrees with Medawar and Williams that natural selection declines with age, but, contrary to their hypotheses that ageing-inducing genes accumulate, considers that selection has favoured longevity assurance (Sacher 1978a) or longevity determinant genes (Cutler 1982). Sacher's (1978a) postulated longevity assurance genes code for regulation, protection and repair of the organism at all levels. Cutler (1980,1982) has postulated a more specific role for longevity determinant genes: stabilisation and maintenance of the differentiated state of the cells of an organism, thereby postponing the dysdifferentiation characteristic of ageing (Ono and Cutler 1978). Thus ageing is the result of this gradual loss of regulatory control over gene expression, and life-span duration is determined by the time of action and degree of expression of longevity determinant genes (Cutler 1982).

Cutler (1978) agrees with Williams that pleiotropic genes with beneficial early effects and deleterious later effects were selected for, but in relation to development not reproduction. The fixation of these pleiotropic genes stemmed directly from the increasing complexity of multicellular organisms and their increasing developmental complexity, which was due to increased cell differentiation and the development of neuroendocrine systems. That is, development and senescence are tied.

Thus the evolution of longer life-span in the higher multicellular organisms with separate soma and germ plasm is achieved by:

1) direct selection for longevity assurance genes; and

2) retarding the rate of development, so that the expression of developmentally linked biosenescent processes are postponed.

Sacher (1982) differs from Cutler in that he does not postulate the existence of developmentally linked ageing processes. The apparently positive correlation between duration of development and life-span in primates, in particular, is the result of increased brain size, which results in selection for longer development time. A longer development reduces the number of progeny, and thus there are selection pressures for a longer reproductive life-span

to restore the intrinsic rate of increase per generation. There is not a genetic correlation between length of life-span and development.

Though Sacher and Cutler differ on this point, their hypotheses are so similar that they are subsumed into the RP hypothesis.

Senescence then is due to a lack of selection for a stable state in the animal after a certain age, both at a genetic and systemic level of regulatory control.

Arking (1987) has developed a model for the genetic regulation of senescence based on data collected from work with *Drosophila* by Clare and Luckinbill (1985), Luckinbill *et al.* (1984) and Luckinbill and Clare (1985). He calls it the "biphasic gene control of ageing" and believes it is compatible with Cutler's theorem that life-span is determined by the time of action and degree of expression of specific longevity determinant genes. However, I would dispute this belief as it appears to me that Arking (1987) has misunderstood Cutler (1978,1982). According to Arking (1987), longevity is controlled via the timing of the onset of senescence. During larval life regulatory genes are influenced by the environment and are "reprogrammed" to either delay or move up the repression of structural genes acting prior to the onset of senescence. Shutting down the processes controlled by these genes brings about the onset of senescence, which is not under genetic control and from this point on is determined stochastically.

However, Cutler (1982) proposes that senescence is the result of a gradual loss of regulatory control over gene expression, not a preprogrammed repression of genes as Arking (1987) suggests. The two mechanisms are quite different though both postulate that senescence is due to running out of genetic program. Arking's (1987) model of ageing appears to be more related to the Developmental than RP hypothesis concerning the evolution of senescence. This is not to say that Arking's (1987) theorem might not have some validity, though there are some theoretical problems with its postulates. Firstly, it proposes a pre-senescent period from early to mid adult life, yet empirical work has shown that many ageing changes gradually accumulate throughout adult life-span (Massie and Williams 1987; Sohal 1985). Such empirical evidence is not suggestive of a pre-senescent adult phase as conceived by Arking (1987). Furthermore, it is difficult to understand why such ageing-inducing genes could have evolved which decrease the fitness of the individual with no apparent benefits.

There has not been much empirical or theoretical work designed to examine the validity of the RP hypothesis. Certainly senescence does seem to be an inherent feature of soma-germ-cell organisms, and linked with developmental complexity. Bacteria do not die but

some of the more complex unicellular organisms age and die. Not all haploid organisms age, but all diploid organisms do (Sonneborn 1978).

Mammalian life-table evolution has been characterised by increased longevity (Sacher 1978a,1982). This increase was a consequence of natural selection for a longer reproductive replacement time. It is easier to believe that such an increase was due to selection for improved repair processes within the organism than the selective elimination of life-shortening alleles which had been already fixed in primitive organisms due to a decreasing force of natural selection with age.

Hart and Setlow (1974) and Sacher and Hart (1978) have shown that DNA repair and replacement are positively correlated with the longevity of mammalian species. Yet a relationship between DNA repair and longevity was not confirmed in cold-blooded vertebrates (Woodhead, Setlow and Grist 1980). However, Woodhead *et al.* (1980) did not examine the mitochondrial DNA which may play a fundamental role in ageing (Massie 1986; Miquel and Fleming 1988). Mitochondrial DNA in many species appears to accumulate senescent damage, leading to a great loss of its information (Massie 1986; Massie and Williams).

There does appear to be derepression of gene expression in mice with age, which may be related to a dysdifferentiation process (Ono and Cutler 1982). It is interesting that a derepression of gene expression during development in *Drosophila melanogaster* results in an initially shortened life-span (Luckinbill and Clare 1985). This appears to link control of gene expression with length of life-span. It also links development and the progress of ageing, as it demonstrates how an alteration in developmental conditions can modify longevity.

There are numerous examples of an epigenetic component to longevity (see section 1.3.3.1), though how large and important this component is, is not yet known. Cutler (1978) has proposed a positive relationship between longevity and development but this was really in reference to between-species comparisons. Since new species may arise as the result of changes in developmental patterns (Arking and Clare 1986), it is perhaps unwise to extrapolate Cutler's hypothesis to within species comparisons within which developmental patterns may be set.

Certainly in *Drosophila,* information about the genetic relationship between developmental duration and longevity is conflicting (Luckinbill *et al.* 1984; Tucić *et al.* 1988). As Arking and Clare (1986) have suggested "changes may be more subtle than a simple increase in total developmental time".

There may be some confusion between this hypothesis and the Unitary hypothesis, especially as Giesel (1979) himself postulated that the positive correlations he found between longevity and early and late life reproductive homeostasis in inbred lines support Sacher's hypothesis of longevity-assurance genes.. Both hypotheses postulate inherent senescence in multicellular organisms. They may be empirically distinguished by their different predictions about the relationship between developmental rate and longevity in natural populations. The Unitary hypothesis predicts that selection for a faster developmental rate should produce more longevous organisms. The Unitary hypothesis would also predict that, because all life-history traits are inextricably bound, any small change in a life-history trait such as reproduction will affect longevity. This is not a necessary corollary of the RP hypothesis.

If the RP hypothesis is correct, then experimental work should show that:

1) it should be possible to directly select for increased longevity; and

2) there should be negative genetic correlations between reproduction and life-span in populations at equilibrium because of strong directional selection on both traits.

Also according to Cutler's hypothesis:

3) disruption of developmental processes should detrimentally affect longevity but, perhaps, expose genetic variation.

But according to Sacher's hypothesis:

4) there should be no genetic link between longevity and duration of development within individuals of a population.

1.3.3.6 Group Selection Hypothesis

Group selection is used to describe any selection that depends upon differential survival or reproduction of groups of individuals (Hedrick 1984). Thus the groups are the evolutionary unit and not individuals. Most cited examples of group selection are dependent upon individual selective values within the group, but there are situations in which traits detrimental to the individual but beneficial to the group, foster the propagation of a group. An example of such a trait may be the alarm-call behaviour in birds and other animals.

Weismann (1891) was the first evolutionary biologist to present a theory of senescence, and he proposed that group selection was the evolutionary mechanism of ageing and death.

He believed that organisms did wear out like machines and that natural selection would act to eliminate the old, and therefore worn-out individuals, that would otherwise compete for limited resources with the younger individuals. Thus a programme for ageing and death would be selected. Obviously selection for such a programme is not beneficial to the individual, but is clearly beneficial to a group composed of young and old individuals.

There are a few examples of programmed death, such as the death of the parents after reproduction in the Pacific salmon, Australian hopping mouse, blowfly, Mediterranean octopus and squid (Collatz 1986; Collatz and Wilps 1985; Hirsch 1979; Kirkwood and Holliday 1979). These programmed deaths are probably mediated by hormones. They may be explained in terms of individual selection theories (Williams 1966a), but it is also possible that group selection was responsible for the evolution of such programmed deaths (Kirkwood and Holliday 1979).

Of course this is not proof enough to invoke group selection as the predominant force in the evolution of senescence, though there have been many other proponents, apart from Weismann such as Wynne-Edwards (1962), Bonner (1974), Denckla (1975) and Leopold (1961,1975).

However, there are a number of strong arguments against group selection as a general mechanism for the evolution of senescence. The theory is somewhat circular in that it presupposes a senescent process in its explanation for the evolution of senescence (Kirkwood and Holliday 1979). If such a process is already in existence then there is no need to postulate a further theory to elucidate its development. But Rose (1983) explained the theory need not be circular, if the "wearing out" is caused by extrinsic factors, such as the accumulation of accidental damage through time. Yet if the group selection theory is correct, and it is an adaptation to remove old organisms, then many organisms should exhibit senescence and die of old age in the wild (Kirkwood and Holliday 1979; Rose 1983a; Williams 1957). Yet very few individuals reach old age in the wild (Comfort 1979), as most normally die from predation, disease or starvation (Lack 1954a). On the other hand, any decrease in viability would increase the likelihood of succumbing to external hazards and the onset of senescence may be all that is needed to eliminate most wild-living organisms (Collatz 1986).

However, it is difficult to imagine how programmed death could be selected for at the group level, when it would always be opposed by selection at the level of the individual for mutations that increase life-span. The complexity and multifaceted nature of the senescence process, also makes it unlikely to be programmed (Collatz 1986).

1.4 Genetic Constraints on Life-History Evolution

1.4.1 Introduction

Genetic constraints are "those genetic aspects that prevent or reduce the potential for natural selection to result in the most direct ascent of the mean phenotype to an optimum" (Loeschke 1987). There are three important classes of genetic constraints:

1) lack of genetic variation;

2) genetic correlations; and

3) developmental processes.

Quantitative genetic analysis can reveal the importance and form of these constraints. The genetic variation of life-history traits is polygenic (Arnold 1981; Ayers and Arnold 1983; Cade 1984; Charlesworth 1984; Istock 1984; Lande 1983; Mitchell-Olds and Rutledge; Primak and Antonovics 1982; Stearns 1980), thus quantitative genetic analysis can be used to analyse the phenotypes in terms of means, variances and covariances, and estimate the quantitative genetic parameters of heritability (h^2) and genetic correlations (r_G) (Mitchell-Olds and Rutledge 1986).

The heritability describes the amount of additive genetic variation for a trait and predicts the expected response to directional selection (Barker and Thomas 1987). The genetic correlation between two traits may be used to predict whether the response to natural selection pressures by one or both of the traits will be constrained or enhanced, and identify sets of coadapted traits, the products of natural selection for "life history tactics" (Etges 1982). Developmental constraints can also be revealed by quantitative genetic studies by analysing the pattern of response to artificial selection (Scharloo 1987) or revealing the inability of a population to respond to repeated and powerful selection (Maynard Smith, Burian, Kauffman, Alberch, Campbell, Goodwin, Lande, Raup and Wolpert 1985).

Other genetic constraints exist but these operate primarily through the process of transmission of genes from parent to offspring and are not amenable to quantitative genetic analysis (see Barker and Thomas 1987), which is the methodology used in this study.

The following sections give a more detailed analysis of the operation of genetic constraints, as well as the relevant genetic parameters, their usefulness and the problems associated with their estimation.

1.4.2 Genetic Variation

If there is no additive genetic variation for a trait, it is unable to respond to selection pressures, natural or artificial. The heritability of a trait is a measure of the amount of additive genetic variation which is present, as it is the ratio of the additive genetic variance to the phenotypic variance. However, there are some problems in using heritabilities as measures of additive genetic variation in natural populations.

Most heritabilities are determined under laboratory conditions which are usually constant environments. Such constant environments minimise the environmental variance and thus heritabilities estimated in the laboratory may be greater than heritabilities estimated under natural conditions (Atkinson 1979; Barker and Thomas 1987; Cade 1984; Prout 1958). There may also be a genotype-environment interaction. Parsons (1977,1978,1982,1987) and Derr (1980) have suggested that additive genetic variance of a trait may increase when a colonising species is stressed, and empirical evidence at this stage supports this hypothesis (Arking and Clare 1986). Transplanting from the wild to laboratory conditions may be such a stress that boosts heritability estimates (Istock 1982; Mitchell-Olds 1986).

Obviously, estimates in the laboratory may differ from those in the wild, but if one is not interested in precise estimates of heritability, a non-zero additive genetic variance in the laboratory may indicate the presence of such variation in the wild (Barker and Thomas 1987). This is assuming a large sample from the natural population and an appropriate experimental design to minimise the standard error of heritability.

In the past it has been assumed, based largely on a misinterpretation of Fisher's Fundamental Theorem of Natural Selection (1930), that fitness components exhibit very little additive genetic variation. Fisher (1930) showed that, under certain idealised conditions, the increase of fitness in one generation is equal to the additive genetic variance of fitness. He proposed that continual directional selection for higher fitness would drive the additive genetic variance for fitness to zero.

It has been suggested by many workers that the idealised conditions and assumptions of the theorem render it inapplicable to the real world (see Barker and Thomas 1987). Perhaps it is not surprising then that substantial additive genetic variation has been found for fitness components in many natural and laboratory populations (e.g. Allen 1984; Berven and Gill 1983; Cade 1984; Dawson and Riddle 1983; Dingle, Brown and Hegmann 1977; Dingle, Evans and Palmer 1988; Emerson, Travis and Blouin 1988; Giesel *et al.* 1982a; Gilbert 1984a,b; Groeters and Dingle 1987; Hegmann and Dingle 1982; Istock 1981; Istock,

Zisfein and Vaura 1976; Jinks and Broadhurst 1963; Mitchell-Olds 1986; Mousseau and Roff 1987; Murphy *et al.* 1983; Perrins and Jones 1974; Riddle *et al.* 1986; Rose 1984b; Rose and Charlesworth 1981a,b; Schwaegerle, Garbutt and Bazzaz 1986; Smith, Sibly and Møller 1987; Tauber and Tauber 1986b; Tucić *et al.* 1988; Van Noordwijk 1984; Wyngaard 1986).

However, one might expect that traits most closely related to fitness might exhibit lower heritabilities than other traits within a population, because of stronger selection pressures (Falconer 1981). Gustaffsson (1986) has found an inverse relation between the heritability of a trait and its influence on fitness. In general, life-history traits have been found to have lower heritabilities than morphological, behavioural and physiological traits (Emerson, Travis and Blouin 1988; Mousseau and Roff 1987; Roff and Mousseau 1987). Components of fitness can have significant heritability values, but fitness itself need not be heritable.

Fitness components are often assumed to have an intermediate optimum (Dawson 1965a), and thus be experiencing stabilising selection (Barker and Thomas 1987). This may be real or spurious i.e. directional selection pressures on antagonistically correlated traits (Barker and Thomas 1987; Falconer 1981). Real stabilising selection is expected to deplete genetic variability (Hedrick 1984; Kaufman, Enfield and Comstock 1977), whereas the effects of spurious stabilising selection are indeterminate (Barker and Thomas 1987). Travis, Emerson and Blouin (1987) have suggested that life-history traits with a high dominance genetic variance component have experienced directional selection and are major components of fitness.

Numerous mechanisms have been postulated as responsible for the maintenance of quantitative genetic variation in fitness components. One is that the combined forces of mutation, which replenishes genetic variability, and stabilising selection maintain observed levels of equilibrium genetic variation in quantitative traits (Kimura 1965; Lande 1976,1977,1980; Turelli 1984,1985). Antagonistic pleiotropy between fitness components, particularly between early and late life-history characters, may also maintain the additive genetic variation even though the genetic variance of fitness itself is zero (Roff and Mousseau 1987; Rose 1982,1983a,b,1985; Smith, Sibly and Møller 1987). Other explanations are genotype by environment interactions and/or fluctuating selection pressures in a temporally or spatially heterogeneous environment (Felsenstein 1976; Giesel *et al.* 1982a; Istock 1978; Mitchell-Olds 1986; Murphy *et al.* 1983; Via and Lande 1985); or, selection for heterozygosity due to pleiotropic overdominance (Gillespie 1984; Lerner 1954a).

Empirical work has not been able to establish which of these mechanisms is responsible

(see Barker and Thomas 1987; Clark 1987b) but it is probably not one but a number at work (Rose, Service and Hutchinson 1987). Other forces which may also play a role in regulating the polygenic variation are genetic drift (Lande 1976), gene flow (Endler 1977); frequency and density dependent selection (Barker and Thomas 1986; Bradley 1982; Bradshaw 1984) and linkage disequilibrium (Cade 1984; Etges 1982).

Lack of genetic variation does not appear to be a major constraint on response to natural selection pressures by life-history traits in most instances. There are other genetically based mechanisms though which may reduce the ability of an organism to respond to selection pressures by modifying the selection pressures on the genotype.

Phenotypic plasticity, the ability of a single genotype to produce a range of environment-dependent phenotypes (Stearns 1982c) is one such mechanism. It has been proposed that phenotypic plasticity frees the gene pool from the impact of selection because the genotype has little relation to the phenotype (Stearns 1982c; Wright 1931). It modifies the selection pressures on the traits, rather than the traits themselves, and may promote genetic stasis in a population (Stearns 1984). Thus, there should be selection for a population to be either phenotypically flexible or genetically variable (Bradshaw 1965), based on the assumption that a given selection regime will select for either one type of variation or the other (Scheiner and Goodnight 1984). However, it has been found that traits that responded most rapidly to selection in mosquitofish were those that were more phenotypically plastic (Stearns 1983b); and that there is no relationship between the amount of plastic variation and genetic variation in natural populations of *Danthonia spicata* (Scheiner and Goodnight 1984). However, this does not mean that an antagonistic relationship between the two might not exist in more uniform environments (Scheiner and Goodnight 1984). Thus in some environments phenotypic plasticity might prevent genetic change, but in others might have no damping effect on the strength of selection.

Genetic variation may not be expressed because of the canalisation of development. Canalisation is the phenomenon that a phenotype remains relatively uniform and "normal" in spite of large genetic and environmental variation (Waddington 1942). Many developmental processes are canalised. This phenomenon is due to selection pressure for the developmental system to reach its normal, possibly optimal, goal in spite of variation. Either a single developmental pathway will be "buffered" against error or selection will maintain many alternative developmental routes all leading to the one phenotypic expression (Rachootin and Thomson 1981). Canalisation can be built up by selection (Scharloo 1987).

Canalisation hides a lot of genetic variation from selective pressures as, firstly, it ensures that many different genotypes produce a single phenotype (Stearns 1982c), and, secondly, by making the trait insensitive to environmental factors (Berven and Gill 1983). A canalised trait has probably experienced strong selection (Berven and Gill 1983). Berven and Gill (1983) found that the expression of traits most closely related to fitness in populations of the wood frog, *Rana sylvatica,* was relatively canalised.

Canalisation can be de-regulated by environmental stress, thus exposing genetic variability (see Arking and Clare 1986). Thus canalisation may buffer an organism against short-term variability but may not be a stumbling block to adaptation to a major change in the internal or external environment.

Developmental buffering systems may also determine the genetic variation exposed to selection as the nature of genetic variation in life-history traits changes with the degree of environmental stress (Derr 1980; Murphy *et al.* 1983; Parsons 1977). For example, Clare and Luckinbill (1985) and Luckinbill and Clare (1986) have shown that under non-stressful conditions alleles controlling longevity in *Drosophila* exhibit dominance, but when organisms are stressed they show near ideal additivity. Suppression of expression of additive genetic variation in non-stressful environments may prevent response to selection pressures, but, since developmental processes can be destabilised by many different types of stressful factors (see Arking and Clare 1986), lack of response should only be a temporary phenomenon. In fact, major environmental changes are probably always stressful for the organism and thus should expose any hidden additive genetic variation.

Bradshaw (1984) has hypothesised that though there does appear to be genetic variation for most life-history traits of most populations, lack of suitable genetic variation might be a constraint following a rapid major environmental change, thus limiting a population's ability to adapt to a new set of environmental circumstances.

The genetic variation itself may also be constrained by genetic correlations.

1.4.3 Genetic Correlations

The genetic cause of correlation is chiefly pleiotropy. Both positive or negative (antagonistic pleiotropy) correlations can constrain responses to selection, dependent upon whether directional selection on the two characters is antagonistic or reinforcing respectively. One cannot deduce a lack of pleiotropy or causal connection between two characters because of a lack of genetic correlation (Cheverud 1984) as positive pleiotropy between two traits may

balance negative pleiotropy. A lack of genetic correlation between two traits though does mean that they are unlikely to constrain each other's responses to natural selection.

A genetic correlation is not always a good measure of commonality of genetic variance between two traits for another reason. It is not only a measure of pleiotropy but also of linkage phase disequilibrium, which is only a transient phenomenon and can be broken by selection (Falconer 1981; Maynard Smith *et al.* 1985). As such it does not act as a genetic constraint and diminishes the value of genetic correlations as measures of genetic constraints. Sib analyses or artificial selection experiments cannot distinguish between linkage and pleiotropy. The only way to avoid or minimise this confounding factor is to use a population which has a low level of linkage disequilibrium, that is a large outbred population of a species with many linkage groups (Cockerham 1956; Weir, Cockerham and Reynolds 1980).

When selection is strongly directional for two correlated traits, as it should be for fitness traits, a large negative genetic correlation between the two is not unexpected, because whilst loci with positive pleiotropic effects will become fixed for the adantageous alleles, loci with antagonistic pleiotropic effects will remain segregating (Rose 1982,1983b,1985; Rose *et al.* 1987). However, if the genetic variance of major fitness components is mainly due to rare recessives, which would be likely to have deleterious effects on more than one component of fitness, the net genetic correlation between the major components might be positive (Falconer 1981). Any local populational subdivision with some level of inbreeding can cause positive genetic correlations among major fitness components.

Genetic correlations also may not be good measures of the genetic constraints acting in natural populations. There is much empirical evidence to suggest that genetic correlations depend on the environment, and genotype x environment interactions affect the genetic correlations (Clark 1987a; van Noordwijk and Gebhardt 1987). Furthermore, natural populations usually experience spatially and/or temporally heterogeneous environments. Genetic correlations across environments cause life-history evolution in any one environment to be influenced by selection acting on life-history in other environments (Groeters and Dingle 1987). Van Noordwijk and Gebhardt (1987) have concluded "that it is dangerous to draw strong conclusions on the importance of negative pleiotropy as a genetic constraint on data gathered from populations in an ecologically unrealistically simple environment".

As a guide to genetic correlations which may act as constraints, sib analyses combined with artificial selection experiments may still be useful, especially if repeated under a variety

of environmental conditions. They will be better guides if the environmental conditions reflect the natural environment(s). Since it is probably impossible to duplicate the full range and combination of natural environmental conditions, extrapolation from the laboratory to nature should always be done with caution. Nevertheless, at least some determination of the genetic constraints acting in a specific environment can be made.

Genetic correlations are important evolutionary constraints but are themselves the product of evolution (Clark 1987b; Loeschke 1987). Theoretical analyses and artificial selection experiments indicate that the variance-covariance matrix may remain fairly constant through time. However, if effective population size is small and/or selection is strong, then they might undergo substantial change (Mitchell-Olds and Rutledge 1986). This is because "there are genes which act on both characters in the same direction, there are genes which act specifically on each character separately and there are genes which act in opposite directions on the two characters" (Scharloo 1987).

However, the relationship between life-history characters could be fixed by natural selection so that only alleles with antagonistic effects or rare deleterious alleles are segregating. Fixed relationships such as these could act as long-term fundamental constraints in natural populations unless a decanalisation of the phenotype occurs in response to strong selection. Gromko (1987) has suggested that constraints imposed by negative genetic correlations may be overcome by selection for modifier genes which influence the pleiotropic effects of an allele separately. Such modifiers, if existent, are probably not able to overcome inherent physiological trade-offs which are based on competing demands on finite resources.

On the other hand, as for genetic variation, novel environments may induce a developmental uncoupling of characters and thus provide a mechanism for physiological adjustments to new environmental conditions (Pashley 1988).

1.4.4 Developmental Constraints

Developmental constraints are defined as a bias in production of variant phenotypes or limitations on phenotypic variability caused by the structure, character, composition or dynamics of the developmental system (Maynard Smith *et al.* 1985; Scharloo 1987). Examples of such constraints are the canalisation of development or the suppression of genetic variation by developmental buffering systems discussed in section 1.4.2. As mentioned before, developmental buffering systems involved in canalisation or suppression of genetic variation can be built up by selection but can be de-regulated by a major mutant or by environmental

stress thus exposing genetic variability. It is not a fundamental property of development systems that cannot be altered (Rendel 1967 in Barker and Thomas 1987).

More fundamental developmental constraints have been postulated (Scharloo 1987). Patterns of development that are characteristic of a taxon may restrict the range of future adaptations in that taxon (Horn, Bonner, Dohle, Katz, Koehl, Meinhardt, Raff, Reif, Stearns and Strathmann 1982; Stearns 1982c). Many developmental processes are hierarchial and depend upon processes occurring at different times as well as at different levels of organisation (Atchley 1984). A change in one process may affect many others, possibly detrimentally.

It is possible that the rules of development constrain the pattern of phenotypic expression for mutants too, even though mutations at the DNA level may be completely random (Cheverud 1984).

Maynard Smith *et al.* (1985) tend to view the genetic variance-covariance matrix of a species as an extension of developmental processes, and thus lack of genetic variation and constraining genetic correlations as proceeding from developmental constraints. For example, Maynard Smith *et al.* (1985) have suggested that a lack of heritable variation for a trait indicates a developmental constraint on the production of future variant phenotypes, whilst genetic correlations "typically reflect causal interrelations during development and may, in fact, depend primarily on the dynamics of the developmental system."

Limitations of developmental systems are probably the basis of all genetic constraints on life-history evolution, but I believe to label every genetic constraint as developmental is too broad a category to be useful when discussing constraints on life-history evolution. Certainly recognition of the developmental component in genetic constraints is useful, but one should also be aware of the selection pressures on a species that have invoked the imposition of constraints.

It is obvious from the above discussion that developmental constraints will be reflected in the variance-covariance matrix of a species (Cheverud 1984), thus quantitative genetic analyses of populations within a species should provide valuable information about all levels of genetic constraints.

Chapter 2

Research Plan

2.1 Synopsis of Experimental Work

Past empirical work has not firmly established that there is a cost to reproduction, nor that the predicted resulting trade-off between early and late reproduction and/or age at death acts as a genetic constraint on the evolution of life-histories. The nature of the genetic variance and covariance matrix of life history characters in a natural population and its effects on responses to natural selection are also in need of clarification. Similarly empirical work has not been able to distinguish between the various theories relating to the evolution of senescence. This is because experimental work has either been poorly designed, limited in scope or used too few numbers to provide much useful information. To contribute towards a better basis from which to investigate these problems, a complete genetic analysis of the life-history strategy of a natural population of *Tribolium castaneum* was undertaken.

The experimental work was as follows:

a) Estimates of genetic variance and covariance components were obtained by a diallel analysis for the major life-history characters, including lifetime reproductive schedule, of a population recently derived from the wild. Information about the relationships between early and late life-history characters was used, firstly, to determine if a cost to reproduction exists and, secondly, to test the predictions of the various theories about the evolution of senescence.

b) Selection for developmental time was conducted in both directions for six generations using the same population of *Tribolium castaneum*. Control lines also were

maintained. In the eighth generation phenotypic parameters were estimated for the life-history characters of all lines. This enabled a check to be made on the efficiency of the predictions of responses to direct and indirect selection based on the results of the initial analysis. Information also was obtained pertinent to the theorem of reproductive cost and to some of the theories concerning the evolution of senescence.

2.2 Experimental Organism

The experimental organism used in this work was the 'rust red flour beetle' *Tribolium castaneum* (Herbst). It is a well known pest of stored cereal products and oil seeds (Haskins 1975) and has been extensively used as an experimental organism in studies of ecology and population genetics (e.g. Bell 1969,1974; Dawson 1964,1965a,b,1968,1970,1975; Mertz 1975; Park 1948; and see Dawson and Riddle 1983). *Tribolium* has many characteristics which make it a useful experimental organism:

a) Populations are common throughout grain growing areas and are technically easy to collect.

b) The diet of wheat flour and dried yeast mixture is uncomplicated, inexpensive and simple to prepare.

c) Optimal conditions of temperature and humidity (33°C and 70% r.h.) are readily provided by incubation.

d) It is not necessary to provide cultures with light/dark cycles as the beetles prefer darkened conditions (Hawk *et al.* 1974), yet exposure to artificial light during examination of the cultures has no effect on development (Chapman 1931).

e) Handling of beetles is simple, as adults can not climb vertical glass surfaces and do not fly below 25°C.

f) Each stage of the life cycle, i.e. egg, larva, pupa and adult, can be readily isolated from the food medium by sifting with meshes of appropriate size.

g) The sex of individuals can be determined prior to sexual maturity at the pupal stage, which has the further advantage in being immobile.

h) The sexing of adults is also a simple process and no etherisation is required, as adults can be immobilised temporarily by cold. Immobilisation by cold has no after-effects on reproductive success or survival.

i) *Tribolium* spp. have high rates of reproduction, short life cycles and long life-spans.

The above traits make it extremely simple to establish and maintain large, outbred populations of *Tribolium* in the laboratory. However, there are some disadvantages to working with *Tribolium,* such as:

a) Adults will cannibalise eggs, pupae and larvae, whilst larvae will eat smaller larvae, eggs and pupae. This is aggravated by high density conditions.

b) *Tribolium* is subject to parasitism.

c) Adult beetles under conditions of high density produce quinones which are mutagenic and may completely destroy a culture (Anon. 1960).

All of these problems can be controlled by maintaining standards of hygiene, changing media regularly, controlling the density of cultures and/or keeping the various life stages separate if necessary.

Tribolium castaneum has other characteristics which make it useful for studies of the genetic variation-covariation matrix of life-history traits. Linkage phase disequilibrium can confound the interpretation of genetic correlations, and to minimise its effects quantitative genetic analyses should be done using large outbred populations, and preferably species that have a large number of linkage groups. *Tribolium castaneum* has nine pairs of autosomes (Anon. 1960; Haskins 1975), so that genetic correlations between traits in large outbred *Tribolium* populations should be primarily due to pleiotropy, and not be overly confounded by linkage. Interpretation of correlations should thus be facilitated when determining if there is a cost to reproduction, and the nature of genetic variation and covariation in natural populations.

A further feature of *T. castaneum* is that laboratory culturing conditions do not differ radically from conditions in the wild. *Tribolium castaneum* is found mainly in cereal grain and grain product storage in silos or stockfeed bins. They are secondary pests requiring that grain be broken before they can colonise, and can attack a wide range of products: "flour and all other prepared cereal products, grain and seed, animal matter especially dry

specimens, yeast, nuts, dried fruits, chocolate, certain spices and other miscellaneous plant products" (Good 1936). The food medium is their habitat (Bergerson and Wool 1986). They are negatively phototactic and respond positively to areas of higher humidity within a food medium (Haskins 1975). Behavioural mechanisms also modify their environmental temperature e.g. they will migrate into the food medium when temperatures are low but come to the surface when temperatures are high.

The microenvironments of silos etc. are usually buffered from the cold, and, especially if they contain large quantities of grain, maintain quite high temperatures for long periods. In Australia, the grain is harvested at near optimal temperatures for growth of grain insect populations (approximately 34°C for *T. castaneum*). Often in such micro-environments the localised temperature is adequate for development and reproduction even though the outside temperature goes below the temperature required for normal development. *Tribolium castaneum* will not lay eggs at temperatures below 20°C and prefers temperatures above 30°C (Orozco and Bell 1974). Therefore food availability is the main limitation on population size and survival of *T. castaneum* in Australia (Johnston 1981).

Thus wild *Tribolium* populations gathered from stored cereal products are adapted to living within a dry medium enclosed in darkness, which are also laboratory conditions. Temperatures and relative humidity are more optimal and constant in the laboratory, but beetles in their natural habitat do experience such conditions and do practise their own kind of environmental control by behavioural mechanisms. Certainly the laboratory environment is a realistic one for the species and beetles are unlikely to find laboratory conditions stressful by their novelty. *Tribolium castaneum* is adapted to meeting "novel" environments anyway as it is a colonising species. Criticisms about using populations recently derived from the wild for quantitative genetic analyses because of the unsettling effects of "novel" environments on the genotype are probably less applicable for this species.

However, it is not expected that freshly caught samples will necessarily accurately reflect genetic correlations of traits in natural populations in their natural environment. Genotype x environment interactions may affect the genetic correlations, particularly in magnitude. However, changes to genetic correlations in a "realistic" environment should not change the nature of fundamental genetic constraints, which are of primary interest to this study.

Tribolium castaneum is of particular interest to experimental gerontologists, in having two features which make them highly suitable for studies on the evolution of senescence. Firstly, though *T. castaneum* is a coloniser and has many of the life-history attributes of

a coloniser: high intrinsic growth rate, rapid development and early maturity, excellent dispersal ability and strong predatory tendencies (Dawson 1977; Mertz 1971), it appears that it has also been selected for deferred senescence (Mertz 1975), an unusual characteristic. It has been postulated that this is probably due to selection under stabilised or decreasing numbers once a colonising episode has been established (Dawson 1977; Mertz 1971). For this reason, *T. castaneum* may be a particularly good experimental organism for an investigation into the Running-out-of-Program theory of senescence.

Secondly, *Tribolium,* in contrast to *Drosophila* (Bozcuk 1972), which is the most commonly used experimental organism in gerontological studies, has somatic cell division occurring in the imaginal stage of the life cycle (Day and Powning 1949; Devi, Lemonde, Srivastava and Sarkar 1963). Most higher organisms show somatic cell division in their adult stages, so that *Tribolium* may be a more suitable frame of reference for the evolution of senescence than *Drosophila.*

One further justification for using *Tribolium* rather than *Drosophila* is simply that so much work has been done using *Drosophila* (see Lints and Soliman 1988), that more information on another organism is required for comparison. Some work on *Tribolium* has been done which provides a good basis for further investigation (e.g. Lavie 1981; Lints 1978; Mertz 1975; Sokal 1970; Soliman and Lints 1975, 1982).

2.3 General Materials and Methods

This section describes the laboratory husbandry procedures used in this study. Since the same population of *Tribolium castaneum* was used in both the diallel analysis and the selection program, its history is included here.

Laboratory Environment. All beetles were maintained in darkened, fan-forced incubators at 33°C and 70%r.h.. These conditions are optimal for egg-laying by *T. castaneum* females (Hawk, Colaianne and Bell 1974; Orozco and Bell 1974) and are standard for *T. castaneum* in laboratories (Sokoloff 1977). Saturated solutions of sodium nitrate ($NaNO_3$) were used to maintain relative humidities of slightly above 70% but this was regarded as advantageous in view of the loss of humidity with the opening of doors etc..

The laboratory temperature and relative humidity were respectively controlled at 21°C and 60-80%. This temperature is below that at which *T. castaneum* will fly, thus ease of handling was ensured. Attempts were made at all times to minimise the length of handling

time beetles spent out of the incubator.

Experimental Containers. In all experiments single beetles or pairs were maintained in 7 ml glass vials with perforated plastic lids, while larger numbers were kept in plastic containers (13.5 x 9.5 x 7.0 cm) with perforated lids. Glass vials can contain up to 2 g of medium, and plastic containers up to 400 g.

The Culture Medium. The culture medium consisted of whole-wheat flour fortified with 5% by weight of dried, powdered brewers' yeast. The dried brewers' yeast powder, supplied by Healtheries Ltd. of Auckland N.Z., was derived from *Saccharomyces cerevisiae.* To maintain constancy of conditions the flour had been obtained from one source before the study commenced. Ben Furney Flour Mills of Dubbo, N.S.W., in February 1984, supplied 550 kg of untreated flour, milled from a blend of 40% NH grade (82-83 pool, Trangie origin, 10.6% protein, varieties: Shortim 35.4%, Banks 34.9% and Cook 29.7%) and 60% A.S.W. grade wheat (83-84 pool, Ballimore origin, 12.7% protein, varieties: Banks 80.0% and Kite 20.0%).

It was sifted through a 210 μm aperture mesh with the aid of Endecott's EVSI variable speed, intermittent motion test sieve shaker, sterilized at 60°C for eight hours (Sokoloff 1977), and then sealed for storage in 7-8 kg lots in sterilized aluminium cans.

Prior to use, flour was thoroughly mixed with the required weight of yeast, measured out into containers and placed into an incubator for conditioning for at least 24 hours at 33°C and 70%r.h.. A Mettler PC 4000 balance was used to measure out quantities of media required for culture containers.

Handling Procedures. As noted above, flour was screened through a mesh aperture of 210 microns to remove any foreign material or organisms, and so that in the future, eggs could be separated and retained from the culturing medium. By sifting with a 210 micron mesh aperture sieve, the presifted medium would pass through but eggs would be retained. To retain adults, pupae and large larvae but not eggs, a 500 μm mesh sieve was used.

Eggs were always counted in a linear egg counting stage which was composed of two glass microscope slides painted black and placed at an acute angle to each other lengthways, to form a "V". The eggs were poured from the sieve into the "V" and counted under low magnification. The stage was constructed following the directions of Muir and Grossman (1973).

Sex Identification. Adult males were identified by sub-basal setiferous punctures on anterior femurs (Hinton 1942), and females were identified by the absence of such "sex pits".

For examination, adults were immobilised by placing them on Plaster of Paris, set in a petri dish, surrounded by crushed ice.

Pupae were sexed by the genital lobes on the ventral surface of the apical segment (Halstead 1963; Haskins 1975). Females have larger and far more prominent lobes than males.

Species Identification. *Tribolium castaneum* may be readily distinguished on the basis of antennal morphology, the degree of ridging of the anterior-dorsal margin of the head, and the relative extent of separation between the eyes (Haskins 1975; Hinton 1942; King and Dawson 1972) from other *Tribolium* species with which it is often found in nature. All flour beetles collected from the wild were checked under a dissecting microscope, and species other than *T. castaneum* were discarded.

Control of Disease and Parasites. To prevent the possible introduction of mites, psocids and transmission of infection by *Farinocystis tribolii* and *Adelina tribolii* to the laboratory, beetles collected from the wild were quarantined by being placed into an incubator outside the laboratory. Eggs from these beetles were washed in 0.1% benzalkonium chloride following the method in Winks (1981). The eggs were then placed into medium *en masse* and transferred to an incubator within the laboratory. All subsequent experimental work was maintained in this incubator.

Hygiene was maintained by sterilizing all metal and glassware at 200°C for at least 60 minutes, and washing all other equipment in hot water and swabbing with ethanol. There were no problems with disease or parasitism throughout the duration of the experiments.

History of Population. A population was obtained from a property called "Echarina" located near Bowna in south-eastern N.S.W.. This population was found in a stock-feed bin which had been undisturbed for years. Because of the age of many of the beetles (rust-red flour beetles become darker with age) and the number of empty body cases in the bin, this population probably had been established for a number of years. Migration was an unlikely source of further genetic variation after the population's foundation as the property is not close to grain growing areas and the population was probably introduced with the feed. Furthermore, other small storages of stock feed brought onto the property at a later date were uninfected by *Tribolium.* As the population was large and *Tribolium* are random-mating and have a large number of linkage groups (Anon. 1960; Haskins 1975), it was probably very close to demographic equilibrium at the time of collection. Thus it can be assumed that heritabilities and genetic correlations between traits are stable for the population in

its natural environment. Though the magnitude of estimates of genetic parameters may have differed between the wild and laboratory environments, steps were taken to minimise changes in the genetic variance-covariance matrix due to selection pressures imposed by laboratory husbandry procedures.

Eight hundred and ninety adults were collected and placed into a plastic container with 200 g medium in the quarantine incubator. The weights of fifty males and fifty females were measured on a Mettler HL 52 balance (reproducibility $\pm$ 0.02 mg) and recorded (Appendix 1).

After a few days of acclimatisation, the beetles were placed into 200 grams of fresh medium. They were removed from the medium after 24 hours and henceforth maintained in the quarantine incubator as stocks.

From the retained media, 2,765 eggs were collected. The eggs were washed, placed into medium *en masse* and transferred to the incubator in the laboratory.

Organisms collected from the wild must be reared through two generations in the laboratory to eliminate in the first generation, first-order developmental plasticity, and in the second generation maternal effects (Stearns 1977). Records of body weights and/or developmental rates of the original adults and the first generation (generations 0 and 1), are summarised and tabulated in Appendix 1; and for generation 2, in Appendix 2.

From day 15 onwards there were daily checks of this generation 1 for pupae. The numbers of pupae emerging daily were recorded and emergent pupae were placed into empty vials to prevent cannibalisation by large larvae.

Cannibalism is rife in laboratory populations of flour beetles (Mertz and Robertson 1970). Adults and larvae devour eggs and pupae, and to a lesser extent other larvae (Craig 1986; Englert and Raibley 1977; Lang and Mertz 1982; Mertz and Robertson 1970; Sokoloff 1974). If pupae were not isolated from larvae the resultant cannibalism would exert a selection pressure for slow developmental rate (Dawson 1975). Complete prevention of cannibalism of pupae may be unnatural, but cannibalism is probably not very prevalent in natural populations which are open to migration and can experience harsh and/or varying environments (Mertz and Robertson 1970; Sokal and Fujii 1972). Thus the prevention of cannibalism of pupae should have few consequences.

The daily check for pupae continued until all "normal" larvae had pupated. Some larvae do not seem to develop beyond a certain stage, and those that had an abnormally slow development were excluded from all experiments. This reduces the incorporation of

deleterious mutations into the population, and also means that adults would not differ by more than two to three weeks in age. The three week age limit was to ensure that there would be no parental age effect on developmental rate and/or longevity of progeny (Dawson 1965b; Soliman and Lints 1975). Less than 0.2% of generation 1 was excluded on this basis.

Four days after the first larvae had pupated, checks for adult emergence began. Imagoes were kept *en masse* in single-sex containers. Once all adults had emerged, 50 males and 50 females were weighed (Appendix 1) and 216 males of 785 males and 216 females of 779 females were randomly selected and set up as single pairs in 1 g medium. The maintenance of the natural population as single pairs minimised random drift (Bray, Bell and King 1962).

Eight days after the last imago had emerged, single pairs were each transferred into 2 g of medium (day 0 of new generation), which is the optimum amount of medium for maximum 24 hour egg lay (Orozco and Ruano 1970). The eight days wait ensured that the youngest beetles should have been fully productive (Erdman 1964) and most females should have attained peak fecundity (Bhat and Bhat 1974b; Howe 1962). *Tribolium castaneum* females have a short preoviposition period (the minimum is 3 days at 32.5°C according to Howe (1962)) which varies in length for individuals. Once oviposition begins, within a few days there is an early flat peak followed by a gradual decline in egg lay rate. Thus the age-specific fecundity curve is typically platykurtic. Egg lay usually continues up to time of death, though there may be a short postreproductive period (Bhat and Bhat 1974b; Howe 1962; Mertz 1975). Adults were removed from the 2 g medium after 24 hours and the vials containing the eggs were retained.

Using a short egg collection period had the advantage that the resulting individuals would all be very similar in age and thus size. Hopefully, this minimised predation by larger larvae on their smaller siblings. The loss of eggs through cannibalism by parents should also have been minimised.

On day 15 daily checks for pupae commenced and the procedure followed for the previous generation was repeated until all adults had emerged (Appendix 2). These generation 2 adults were used as the parental generation for the diallel analysis and to establish a control population whose later generations were used in the selection experiment.

Specific details of the procedures of each experiment are given in the relevant chapters.