Chapter 1

Introduction

This chapter summarizes the available theoretical and empirical literature relevant to the evolution of life-histories. It is not intended to be a comprehensive critique of the area, but rather to set the scene for the experimental studies reported in the following chapters. Together the fields of ecology and genetics can describe the adaptation of an organism to its environment through evolution by natural selection. Consideration of one field in ignorance of the other would surely prove unproductive.

1.1 Natural Selection as the Major Process in Evolution

In any study concerning evolution in natural populations it is necessary to define and discuss the relationship between evolution and the process of natural selection.

Evolution is defined as "any net directional change or any cumulative change in the characteristics of organisms or populations over many generations" (Endler 1986). Further, it explicitly includes the origin as well as the spread of alleles, variants, trait values, or character states (Endler 1986; Mayr 1963).

It was Darwin in 1859 in "The Origin of Species" who introduced and defined the concept of natural selection — "this preservation of favourable individual differences and variations, and the destruction of those which are injurious, I have called Natural Selection, or the Survival of the Fittest".

In much of the literature, it is natural selection which is often taken to be the directive force of evolution, in the sense that it specifies the non-random conditions necessary for the evolution of adaptations (Byerly 1983). However, migration, random genetic drift and

mutation are also factors, or primary evolutionary forces, which can affect the genetic constitution of a population (Grant 1985; Hedrick and Murray 1984). Unlike natural selection, mutation, migration and genetic drift are processes which change gene frequencies independently of whether or not such changes increase or decrease the adaptation of organisms to their environments (Ayala 1982).

During the development of the Modern Synthesis, sometimes called neo-Darwinism, there have been various definitions and descriptions of natural selection including: "the differential perpetuation of genotypes" (Mayr 1963); "the differential reproduction of alternative genetic variants, determined by the fact that some variants increase the chances of survival and reproduction of their carriers relative to the carriers of other variants" (Ayala 1982); "the differential viability and fertility of different members of a population as a result of their different degrees of adaptation to the environment" (Mayo 1983); and "the process of adaptive evolution in terms of variation between individuals in their capacity to contribute progeny to future generations" (Mackay 1985).

More recently, Endler (1986) has defined the process of natural selection in the form of a syllogism. Natural selection inevitably occurs within a population given three biological conditions:

a) that individuals vary in some trait or attribute (*variation*);

b) that a consistent relationship exists between that trait and mating ability, fertilizing ability, fertility, fecundity, and/or survivorship (*fitness differences*); and

c) that the trait is heritable and its inheritance is at least partially independent of common environmental effects (*inheritance*).

These conditions, which are necessary and sufficient for the process of natural selection to occur, lead to two deductions:

1) trait distributions will differ among age-classes or life-history stages, beyond that expected from ontogeny; and

2) if the population is not at equilibrium, there will be a predictable difference among generations in trait distributions, beyond that expected from a knowledge of variation and inheritance alone.

Therefore, as a result of this process the trait distributions may change in a predictable way over many generations.

In quantitative genetics the process of natural selection, by nature of its definition, is often broken down into the sequential subprocesses of phenotypic selection and genetic response (Falconer 1981). Phenotypic selection which requires variation and differential fitness, after Endler's (1986) definition, can theoretically be independent of any genetic system. The genetic response is the genetic change that results from phenotypic selection acting on the underlying genetic system. Some authors, like Lande and Arnold (1983) have ignored the genetic response in their use of the term natural selection. They consider natural selection to act on phenotypes, regardless of their genetic basis, producing immediate phenotypic effects within a generation. However, natural selection, as defined by Endler (1986), cannot occur without both subprocesses.

Since the process of natural selection is dependent upon "fitness differences", it is important to establish what is meant by fitness if it is to be used as a "description" (Endler 1986) of the "character" (Falconer 1981) on which natural selection acts. However, defining fitness is not an easy matter. Such comments as "it is not clear how fitness is best defined" (Istock 1982) and fitness is "something everyone understands but no one can define precisely" (Stearns 1976), highlight this problem. Nevertheless, despite ongoing argument about the exact definition of fitness and its measurement, it has been the basis upon which the field of evolutionary biology has been built (Haymer and Hartl 1982).

Following are several definitions of the term fitness, which give some indication of its usage and interpretation. Falconer (1981) has defined the fitness of an individual as "the contribution of genes that it makes to the next generation, or the number of its progeny represented in the next generation". Stearns (1976) considers fit organisms "as those better represented in future generations than their relatively unfit competitors". Endler (1986) defined fitness as "the degree of demographic difference among phenotypes", i.e. fitness can be measured by the average lifetime contribution to the breeding population, by a carrier of a phenotype or of a class of phenotypes, relative to the contributions of other phenotypes. Christiansen (1984) simply refers to fitness as "the character of the individual on which natural selection acts". To empirically examine this "character" it is usually necessary to break fitness down into individual components, as measuring the major components of fitness separately is less difficult than measuring total fitness directly (Falconer 1981).

The two major components of fitness are the total number of offspring produced and the quality of these offspring (Falconer 1981). These major components can be in turn attributed to other characters such as survival, viability, fertility and fecundity (Grant

1985). How these components interact in determining the total fitness of an individual is a difficult question to answer and open to contention. Theoretically, overall fitness can be estimated by combining the values of the separate components (Ayala 1982; Doyle and Myers 1982; Falconer 1981; Grant 1985; Hiraizumi 1985; Lande and Arnold 1983; Mackay 1985). However, it can be misleading to infer total fitness from the individual components (Gustafsson 1986; Haymer and Hartl 1982; Milkman 1983; Prout 1971; Yamazaki and Hirose 1984) since it is the overall, or net fitness, and not the individual components which is important to the process of natural selection. Nevertheless, accurate estimates of the major components of fitness will give valuable insights into any study on natural selection, provided such components are highly correlated with fitness, and any possible interactions between the components have been considered.

The importance of the process of natural selection to evolution, and to the maintenance of genetic variation is a matter of opinion and considerable debate. This dilemma has led to the philosophical problems of interpreting the principle of natural selection as a law. One reason for being interested in construing natural selection as a law is to clarify the scope and function of the process in evolutionary theory (Byerly 1983). Reed (1981) argued that natural selection is a "law of nature" and "because natural selection is a law of nature it is exceptionless; hence, although other processes are involved in evolutionary change, natural selection itself is always and everywhere involved as well". Even though a clear distinction between the explanatory power of natural selection and its universal applicability has been made by Reed (1981), natural selection is not a fact but an hypothesis, a law that should be validated (Krimbas 1984). Byerly (1983) discussed the problems associated with interpreting the process of natural selection as a law, and suggested that the formulation of natural selection as a "framework principle" could contribute to a better development of evolutionary theory.

No modern biologist doubts that evolution has and continues to take place, and few would doubt that natural selection is by far the most important process (Deshmukh 1986). Nevertheless, it is possible for the process of random genetic drift to cause genetic changes in populations by chance alone. It is on this basis that the "neutralist-selection" controversy has arisen.

The "selection" view proposes that natural selection is the most important factor in evolution, and that genetic variability is maintained mainly by balancing, stabilizing, and geographically varying selection, which may shift as the environment changes. Although

mutation is regarded as the ultimate source of genetic variation, its role in evolution is considered to be minor. The "neutral" theory on the other hand purports that at the molecular level most evolutionary change and most of the variability within species are not caused by selection but by random drift of mutant alleles that are selectively neutral or nearly neutral, but not necessarily functionless. It is not the purpose here to review these and other views (see Crow 1985; Endler 1986; Kimura 1983; Lewontin 1974; Nei 1983), but merely to indicate that they differ on the relative effects of selection and genetic drift on variation as well as in evolution.

In conclusion, evolutionists have yet to determine the nature and extent of the role played by natural selection in the evolutionary process. While direct natural selection of character states may be a sufficient explanation of evolution, it is not a necessary one and other forces and phenomena may be causes of evolution (Lewontin 1986). In comparative studies of phenotypes, especially life-histories, explanations for between population and/or between species differences are often phrased in terms of differential adaptation. Such explanations are sometimes accepted without considering alternative non-adaptive hypotheses (Lynch and Hill 1986). In this thesis, natural selection will be considered to be the primary process in the evolution of life-histories, but always with the reservation that random or non-adaptive processes could offer alternative explanations.

1.2 Life-Histories and Their Importance

Life-history theory, since its conception in a paper by Cole (1954), "has developed into a complex field with many hypotheses, approaches, models, and assumptions" (Giesel, Murphy and Manlove 1982b). Stearns (1976) attempted "to bring order to a field whose natural complexity has been compounded by a proliferation of viewpoints". Obviously, the difficulty facing all studies concerned with the evolution of life-histories is sifting through the numerous theories which abound in the field. In this section, the definition and meaning of "life-history" will be explained and its subjection to the process of natural selection and evolution established.

A life-history has been narrowly defined by Law (1979) as "sets of age-specific rates of reproduction and risks of death" or more broadly defined by Lande (1982b) "to include not only the age-specific fecundity and mortality rates, but the entire sequence of changes through which an organism passes in its development from conception to death".

The theory of life-history has developed around the idea that the suite of characteristics that compose a life-history have been "coadapted" and "designed" by natural selection to solve particular ecological problems encountered in particular environments — a tactic (Stearns 1976). Generally, the words "tactic" and "strategy" are used as a shorthand in ecology and evolutionary biology (and in this thesis) to depict the understanding that life-history traits are under the influence of natural selection and undergo evolutionary change (Price 1984). At other times, the word "strategy" has been used as a synonym for phenotype (Eshel 1982). As with other complex adaptations, life-histories consist not of single characters, but of sets of phenotypic traits that covary and function together (Dingle, Blau, Brown and Hegmann 1982; Frazzetta 1975). However, it may prove misleading to accept without empirical evidence that such coadaptation or covariation of life-history characters occurs in all, if any, life-histories (Istock 1984; Stearns 1976).

The idea that life-history features of organisms are subject to the process of natural selection received its main impetus from Cole (1954) — "it is to be expected that natural selection will be influential in shaping life-history patterns" and "that such features observed in existing species should be considered adaptations". It has been established in the previous section that the process of natural selection is dependent upon differential fitness. The two components most frequently associated with definitions of fitness are those of differential reproduction and survival. Since Rose (1983a) considers a life-history to be composed of only two types of character, those of survival and reproduction, it then becomes clear why fitness components are considered by many authors to be synonymous with life-history characters (Denno and Dingle 1981; Dingle and Baldwin 1983; Dingle and Hegmann 1982; Istock 1981, 1982, 1983; Rose and Charlesworth 1981a; Rose, Service and Hutchinson 1987; Stearns 1982b).

This coupling of the life-history with evolutionary change means that genetic variation for life-history characters is fundamental to evolution. Therefore, in attempting to find an acceptable theory of life-history evolution we are left with a theory which is "tantamount to neo-Darwinism, nothing less" (Rose 1983a). Hence the direct measurement of the amounts and kinds of heritable variation for life-history characters present in natural populations can be one of the most meaningful assessments of the raw material upon which natural selection acts (Istock 1984).

In life-history theory, one studies how phenotypic traits interact to affect some measure of fitness (Stearns 1982b). What characteristics influence reproduction and survival are

obviously dependent upon the biology of the organism or species in question. Nevertheless, a life-history strategy usually consists of at least the following components: juvenile and adult mortality schedules; age at first reproduction: reproductive lifespan: age-specific fecundity and fertility schedules; the degree of parental care which includes any parental activity increasing survival of eggs or young; and reproductive effort (Wilbur, Tinkle and Collins 1974).

Flexibility of response to environmental uncertainties can also be an important element in life-history evolution (Dingle 1984). Environmental changes have three general characteristics that are relevant to organisms -- magnitude, predictability and duration. Insects adapt to changing environments in a number of highly characteristic ways. Insects can cope with short-term aseasonal fluctuations in their environment primarily through physiological and behavioural adaptations which facilitate survival. Such adaptations involve aseasonal quiesence, aseasonal migration and aseasonal polyphenism (Tauber, Tauber and Masaki 1986)

In contrast, adaptations to seasonal environmental fluctuations may involve dormancy, seasonal migration and seasonal polyphenism which are usually interrelated through the phenomenon of diapause. Most insects have evolved the ability to perceive environmental cues that signal oncoming seasonal changes, and they respond to such cues by undergoing specific behavioural, physiological, and morphological modifications that prepare them for approaching adverse conditions. Seasonal adaptations are more concerned with overall timing of the whole life cycle to seasonal changes in the environment, rather than just survival (Tauber et al. 1986).

These complex adaptations, as a result of seasonal environmental uncertainties, and life-history or fitness traits are interrelated, so that selective pressure on one causes, to varying degrees, selection pressure on the other. Thus, when the evolution and expression of life-histories are considered, these adaptations emerge as important strategies affecting life-history patterns (Begon and Mortimer 1986; Dingle 1984; Hedrick 1984; Tauber and Tauber 1982, 1986a,b; Tauber et al. 1986).

The consideration of longevity in the same evolutionary light as other life-history characters has evoked much controversy in life-history theory (see Luckinbill and Clare 1986). Since death is the culmination of senescence, longevity is largely dependent upon the rate of senescence. The problem raised by senescence, and consequently longevity, for evolutionists has been to devise a credible theory to account for the establishment and maintenance by

natural selection of this apparently deleterious phenomenon (Charlesworth 1980).

At the individual level senescence is due to the failure of many different physiological functions which affect an organism's viability, and in turn decrease its ability to re-establish homeostasis in response to environmental perturbations (Comfort 1979; Sohal 1985). At the population level, the most concise definition of ageing is "that the overall process of progressive, generalized impairment of the functions of organs and tissues results in an increasing age-specific death rate" (Kirkwood and Holliday 1986).

Although evolutionary theories of senescence differ in the mode of action by which genes are postulated to control the ageing process, they all view the rate of senescence as an adaptable feature of the life-history and therefore modifiable by natural selection (King 1982; Luckinbill, Arking, Clare, Cirocco and Buck 1984; Luckinbill and Clare 1986; Rose 1984c, 1985b; Rose and Charlesworth 1981a,b). Senescence can be viewed not as a pathological condition but rather as one component of the life-history of an organism (Mertz 1975).

The number of reproductive episodes within a lifetime is a key life-history character. Reproduction as a function of age can be distinguished by two fundamentally different patterns. These were termed semelparous and iteroparous life-histories by Cole (1954). Semelparity refers to those organisms whose reproduction is confined to a single age-class and followed by death ("big-bang" reproduction), whereas iteroparous organisms reproduce, or are capable of reproducing, more than once in a lifetime (repeated reproduction) (Bell 1980; Watkinson and White 1985).

In this section I have defined what a life-history tactic or strategy is and some of the great variety of traits and specific adaptations which make up such a phenomenon. It must be remembered that a life-history includes "the entire sequence of changes through which an organism passes in its development from conception to death" (Lande 1982b) and "each individual, each population and each species must, clearly, have its own unique life-history strategy" (Begon and Mortimer 1986). The theory of life-history attempts to predict what traits will evolve and in what way in populations under the influence of natural selection in any particular real situation (Stearns 1976). Therefore, not only is it desirable to categorize or systematize life-history phenomena, but also to elucidate the evolutionary processes that shape particular life-history patterns, and thereby better understand their origins, course of evolution, and adaptive properties (Istock 1984).

1.3 Comparative Studies: At What Taxonomic Level?

An underlying assumption pervading much of life-history theory is that at least part of the variation in life-history traits observed within and among populations is genetically based and represents local adaptation to specific environments (Wyngaard 1986a). It is generally assumed that the process of natural selection is the most important factor producing such adaptation (Stearns 1976).

Few organisms, though there are some (see Endler 1986 for examples), evolve sufficiently rapidly in nature to provide an opportunity to observe the evolution of life-history traits. An alternative is to examine geographically isolated populations that have presumably undergone evolution and genetic differentiation in response to their separate environments (Wyngaard 1986b). This comparative approach to the study of life-histories attempts to relate observed differences in life-history traits to variation in environmental factors, and thereby gain insight into the nature of selective forces acting to shape the components of fitness over evolutionary time (Baldwin and Dingle 1986; Berven 1982a,b, 1987). When comparing existing forms or populations, it is assumed that the conditions under which these forms or populations are living represent the conditions under which they evolved (Stearns 1977).

The most common method used to detect the influence of natural selection on life-histories in natural populations has been to correlate variation in life-history traits with variation in environmental factors. This method is based on the assumption that if natural selection occurs, then geographic variation in the selective factor will give rise to parallel geographic variation in fitness traits (Endler 1986).

It must be emphasized that a correlation may suggest a causal relationship, but it is not sufficient to demonstrate it. In other words, this method does not directly demonstrate evolution by natural selection, and it can only be postulated that natural selection is responsible for the observed patterns of variation.

The different taxonomic levels at which patterns in life-histories can be detected or compared are: interspecific comparisons between closely related species; intraspecific comparisons among populations of the same species; and intrapopulation comparisons between individuals of the same population. It is the purpose of this section to give appropriate examples for each level of comparison and to determine which, if any, is the more appropriate taxonomic level for comparing life-history "strategies".

1.3.1 Interspecific Comparisons

Stearns (1980) suggested that broad surveys of life-history traits across genera, families and even phyla were more likely to perceive life-history "tactics" than intraspecific comparisons where the life-history may be constrained by allometric relationships, be subject to complex interactions with physiological, developmental, or behavioural variables, or be subject to genetic constraints. This was in contrast to the request for detailed intraspecific comparisons made in an earlier paper (Stearns 1976). At higher taxonomic levels, more unlike organisms are being compared, and therefore, more differences in life-histories are expected (Brown 1983).

Stearns (1977) summarized the diversity of life-history traits for many taxa by listing papers making interspecific comparisons among salamanders, lizards, birds, mammals, fish, insects and herbaceous flowering plants. Since then, examples of interspecific comparisons have included those among parasitoids in the families *Ichneumonidae* and *Tachinidae* (see Price 1984); daphnids (Bengtsson 1986; Foran 1986); water striders (Vepsäläinen and Patama 1983); flour beetles (Wu 1981); milkweed bugs (Dingle and Baldwin 1983; Dingle et al. 1982); cavernicolous *Ptomaphagus* beetles (Peck 1986); freshwater crustaceans (Lynch 1980); freshwater snails (Brown 1983); amphibians (Kuramoto 1978); squamate reptiles (Dunham and Miles 1985; Stearns 1984); finches (Price and Grant 1985); birds and mammals (Western and Ssemakula 1982); and mammals (Gittleman 1986; Harvey and Clutton-Brock 1985; Millar and Zammuto 1983; Stearns 1983e).

Brown (1983) compared life-history "tactics" at several taxonomic levels in freshwater snails, using data from the literature grouped according to the type of habitat. The rationale was that tactics should differ among habitats, if selection regimes differ among them. Contrasts in life-history tactics were more evident at higher taxonomic levels as compared with the population level, supporting the prediction of Stearns (1980). However, the reasons for the greater contrasts at higher taxonomic levels were not easily understood — the differences observed were more likely to be confounded with factors other than adaptation to divergent selection regimes (Brown 1983).

Stearns (1984) analyzed the patterns of covariation in five life-history traits within the squamate reptiles (lizards and snakes), also using data from the literature. An earlier paper (Stearns 1983e) examined patterns of covariation in similar life-history traits among mammals. The impact of body size and phylogeny on patterns of covariation in life-history traits were stronger in the mammals than in the reptiles, but remained significant in the

reptiles and led to similar conclusions. Stearns (1984) concluded that the impact of size and lineage on patterns of covariation in the life-history traits of reptiles suggested that microevolutionary explanations, such as adaptation to local environmental conditions, while perhaps necessary, were insufficient to account for the patterns in the data.

Unfortunately, the analysis of patterns of covariation among life-history traits within the squamate reptiles made by Stearns (1984) was seriously flawed. Vitt and Seigel (1985) have detailed a number of errors in both the data and the analysis — errors acknowledged by Stearns (1985). There were five categories of errors evident: spelling of scientific names, assignment of species to higher taxa (families), use of scientific names, extraction of data from papers cited, and citations. Vitt and Seigel (1985) were not criticizing the theoretical approach used by Stearns (1984), but rather were pointing out that with so many errors in the data set, the validity of the conclusions were at best questionable.

Dunham and Miles (1985), using an expanded and corrected data set, and improved statistical procedures, quantified the effects of constraints imposed by body size and taxonomic position (phylogeny) on the patterns of covariation in life-history traits within the squamate reptiles. Their findings of significant effects of family-level taxonomy on four life-history variables, with the effects of size removed, suggested that comparisons within the squamates should be limited to, or below, the family level. Further, the within-family patterns of covariation revealed by the analysis, suggested that local adaptation, plasticity of response to local environmental heterogeneity, and physiological constraints were likely to be important determinants of life-history variation. Although the studies of Stearns (1984) (ignoring the errors) and Dunham and Miles (1985) differ with respect to the appropriate taxonomic level useful in making comparisons in patterns of life-history variation in reptiles, the importance of phylogenetic effects in analyses of life-history evolution has been demonstrated.

Wootton (1987) examined age at first reproduction of 547 mammalian species to determine the influence of diet and habitat on the evolution of life-history traits. The results suggested that body mass, or some correlated factor, and phylogeny strongly constrain age at first reproduction, with ecological factors appearing to have little effect on the evolution of age at first reproduction.

Harvey and Clutton-Brock (1985) discussed some of the problems faced in making interspecific comparisons. The quality of data is inevitably variable in any interspecific comparison. Closely related species tend to be very similar in most measures, and the inclusion of

many similar species from the same genus can inflate sample sizes and produce significant results which are misleading. If general conclusions are to be drawn, it is important that data included in comparative analyses be widely distributed across taxonomic sub-categories. Since the distributions of many life-history variables are highly correlated, problems arise in multivariate analyses and interpretation of results. Further, because the distribution of statistical error is not known in comparative studies of this sort, it is difficult to choose among regression models. Also, comparing the results of different life-history studies is complicated since "methodologically unsound analyses are commonplace" (see Harvey and Clutton-Brock 1985).

While comparisons among higher taxa are valuable in identifying trends in evolution and in suggesting forces of natural selection that might operate during the evolution of life-histories, studies at the intraspecific level are necessary to reveal the available genetic variation existing within and between populations, since it is upon this variation that natural selection acts (Dearn 1977; Dingle et al. 1982). Further, the differences observed at higher taxonomic levels are more likely to be confounded with factors other than adaptation to divergent selective regimes (Brown 1983). Even two ecologically similar species, *Tribolium castaneum* and *Tribolium confusum*, can have very different life-history "tactics" (Wu 1981).

1.3.2 Intraspecific Comparisons

The foundation of most evolutionary theory rests upon inferences drawn from geographic variation or upon the verification of predictions made about it (Gould and Johnston 1972). "Population differentiation within species is universal" and "any character can be affected" (Bradshaw 1984), life-history characters included. Intraspecific studies compare life-history characteristics between geographically isolated populations of the same species that have presumably undergone evolution and genetic differentiation in response to their different environments (Wyngaard 1986a,b). The important implication here is that intraspecific life-history variation provides the opportunity for monitoring the dynamics of life-history evolution within a natural ecological context (Venable 1984).

To the statistician, the term population usually refers to the totality of individual observations about which inferences are to be made, and existing within a definitely specified sampling area in space and time. To the geneticist, a population is usually defined as a group of organisms that share a common gene pool (King and Dawson 1972). This definition lacks ambiguity provided individuals in the population are bisexual, randomly mate

throughout the population or are restricted to a single homogeneous environment, and are free from external gene flow. Even though these criteria are seldom met in nature the definition is usually adequate. The genetic information contained within the gene pool of a population is at any instant in time distributed among a group of individuals as their genotypes. These individuals are merely "temporary custodians" of the genetic information which is redistributed with each succeeding generation (Crawford 1984).

Life-history traits, being the major determinants of fitness, should be sensitive to variations in selection pressures over the range of environments a species encounters in nature (Bell 1980). Should a particular environmental factor affect the relative fitness of different genotypes, a correlation between that environmental factor and gene or genotype frequencies may be expected (Mulley, James and Barker 1979).

The methodology behind intraspecific comparisons can be expressed in the form of a null hypothesis and two alternative hypotheses, either of which may be accepted if the null hypothesis is rejected (after Endler 1986 :

Null hypothesis	H_0:	geographically separate populations are not genetically differentiated.
Alternative hypotheses	H_{A1}:	the populations are genetically differentiated, having adapted particular life-history tactics to particular environmental regimes, due to the influence of natural selection.
	H_{A2}:	processes other than natural selection are responsible for the differences between populations.

Stearns (1980) has questioned whether natural selection acts at the intraspecific level to form sets of coadaptive traits that are designed to solve particular ecological problems. Recent empirical work, however, has documented considerable intraspecific life-history variation in a range of species. These studies of geographic variation in life-histories have usually focused on comparisons between populations separated by large distances, or between populations from radically different environments.

Some examples of intraspecific studies showing population differentiation include those

among populations of *Drosophila serrata* (Birch, Dobzhansky, Elliot and Lewontin 1963); *Drosophila melanogaster* (Giesel et al. 1982b); the Queensland fruit fly, *Dacus tryoni* (Bateman 1967); the pitcher-plant mosquito, *Wyeomyia smithii* (Bradshaw 1986; Istock 1981); the milkweed bugs, *Oncopeltus fasciatus* (Baldwin and Dingle 1986; Dingle 1981; Dingle and Baldwin 1983; Dingle et al. 1982; Groeters and Dingle 1987), *Oncopeltus cingulifer* (Dingle and Baldwin 1983) and *Lygeaus kalmii* (Dingle et al. 1982); the pea aphid, *Acyrthosiphon pisum* (Lamb and MacKay 1983); the Colorado potato beetle, *Leptinotarsa decemlineata* (Hare and Kennedy 1986); the lacewing, *Chrysoperla carnea* (Tauber and Tauber 1986a,b); the freshwater copepod, *Mesocyclops edax* (Allan 1984; Wyngaard 1986a,b); the fingernail clam, *Pisidium casertanum* (Bailey and Mackie 1986); the freshwater leech, *Erpobdella octoculata* (Maltby and Calow 1986a,b); the mosquitofish, *Gambusia affinis* (Stearns 1983a); Trinidadian guppies, *Poecilia reticulata* (Reznick 1982; Reznick and Endler 1982); the wood frog, *Rana sylvatica* (Berven and Gill 1983); the grasses, *Poa annua* (Law, Bradshaw and Putwain 1977) and *Danthonia spicata* (Scheiner and Goodnight 1984); the partially self-pollinating herbaceous annual, *Impatiens capensis* (Mitchell-Olds 1986); and the winter annual plants, *Geranium carolinianum* (Roach 1986) and *Phlox drummondii* (Schwaegerle, Garbutt and Bazzaz 1986). A survey of the literature by Venable (1984) on plant life-histories, indicated that intraspecific variation exists for a wide variety of traits including germination, age-specific survivorship, fecundity, finite rates of increase, seed bank dynamics, seed yield and yield components, and module dynamics.

A lack of significant life-history variation at the population level in the pond snail *Lymnaea elodes* (Brown 1985) was consistent with the earlier comparative study of the literature on life-history variation in freshwater snails (Brown 1983). Much of the intraspecific variation in life-histories observed in this species is considered the result of phenotypic plasticity.

Via (1984a) found that populations of the polyphagous herbivore *Liriomyza sativae* originating from tomato (*Lycopersicon esculentum*) and cowpea (*Vigna unguiculata*) crops differed very little in average responses to the two host plant species, indicating that population divergence had not occurred in this system. However, the absence of "host races" in this study may have been due to frequent migration among crops, given the close spatial proximity of the test fields and yearly crop rotation. These two examples indicate that population differentiation may not always exist, or at least may remain undetected.

In addition to intraspecific comparisons between populations separated by large distances or between populations from radically different environments, comparative studies

of life-history features among populations distributed over clinal gradients are particularly informative when attempting to determine the selective forces which influence certain characters and the adaptive significance of particular strategies (Benton and Uetz 1986; Dearn 1977).

Clinal variation, simply defined, is a gradient in a measurable character (Huxley 1942), or a spatial gradient in the frequency of a gene, genotype or phenotype (Endler 1977). Further, it is a form of geographic variation commonly observed in natural populations (Baumgartner 1986; Mayr 1963).

The study of clinal variation is usually motivated by a desire to demonstrate a relationship between natural selection, the environment and the genotype, since clines can be produced by differential selection, either along a continuous environmental gradient or across abrupt environmental discontinuities (Pearse and Murray 1981). If environmental factors which vary over the range of a given species determine the relative fitness of genotypes, then correlations may be expected between a character gradient and a gradient in the environment (Tomaszewski, Schaffer and Johnson 1973). While there have been various opinions expressed as to the proper interpretation of geographic variation, or lack thereof, it is generally conceded that when patterns of genetic variation are associated with corresponding patterns of environmental variation, there is at least a strong suggestion that the genetic variation in question is adaptive, and the result of the process of natural selection (Kojima, Smouse, Yang, Nair and Brncic 1972; Mayr 1956; Tomaszewski et al. 1973).

Natural selection can contribute to the formation of a cline if there is geographic variation in some environmental factor, and the fitnesses of particular genotypes alter with changes in the environmental factor (genotype-environment interaction). Given an environmental gradient, populations initially similar in their character state distributions will diverge along the gradient forming a cline (Endler 1983). This is called primary contact because the populations are continuously in contact during the evolution of the cline. Secondary contact of two formerly isolated groups of populations, if the groups had differing character state distributions before the contact, can also result in the formation of a cline. The divergence of the characters during isolation could have resulted from past ecological differences or by chance. If there is an environmental gradient along a cline which resulted from secondary contact, the cline will reach an equilibrium between natural selection favouring differences among populations, and dispersal or gene flow, making the populations more uniform (Endler 1983; Mayr 1963).

In most studies, however, it is difficult to interpret observed clinal patterns unambiguously in terms of natural selection, since several other processes can also produce and maintain clinal variation. For example, clines caused by genetic drift and migration may, by chance, parallel environmental gradients and erroneously suggest adaptive relationships (Mulley et al. 1979).

Gene flow, defined by Endler (1977) as "the movement of genes and gene complexes into, and their establishment in, allochthonous gene pools", may counteract the factors that favour geographic differentiation among populations. Gene flow is distinguished from migration and dispersal, neither of which necessarily leads to the entry of gene or gene arrangements into a given gene pool. Endler (1977) defines migration as "the relatively long-distance movements made by large numbers of individuals in approximately the same direction at approximately the same time", whereas, dispersal is "the roughly random and non-directional small-scale movements made by individuals rather than by groups, continuously rather than periodically, as a result of their daily activities". Further, over smooth environmental gradients gene flow is expected to prevent anything but smooth clines.

One of the most persuasive pieces of evidence that natural selection is acting on the genetic structure of natural populations is the occurrence of latitudinal clines (Capy, David, Allemand, Hyytia and Rouault 1983). Clines associated with latitudinal gradients are often used to demonstrate the adaptive value of a trait and to indicate the nature of environmental factors responsible for such adaptations in natural populations (Allemand and David 1976). However, speculation on the adaptive significance of any geographic variation is complicated by the fact that much of the genetic component of variation observed in a particular trait may be the result of natural selection by more than one climatic, habitat, or biotic factor (Power 1969). Life-history traits, and strategies if they exist, are no exception, and will of course, not depend simply on the physical environment as crudely reflected in the latitude, but will also involve all manner of behavioural and ecological considerations (May and Rubenstein 1985).

Lonsdale and Levinton (1985) demonstrated the presence of significant differentiation in three life-history characters (developmental rate, adult body length, and somatic growth rate) in the estuarine harpacticoid copepod *Scottolana canadensis* collected from a broad range of latitudes and reared in the laboratory for several generations under the same conditions. Temperature was suggested as being the principle environmental variable affecting

these latitudinally related physiological adaptations.

Many other studies have detected latitudinal clines in a variety of species and range of traits. Some of the more detailed have reported latitudinal clines for body size in *Drosophila robusta* (Stalker and Carson 1947); morphology (Misra and Reeve 1964; Prevosti 1955) and chromosomal arrangements (Prevosti, Serra, Ribo, Aguade, Sagarra, Monclús and Garcia 1985) in *Drosophila subobscura*; fresh body weight, ovariale number and ethanol tolerance in *Drosophila simulans* (David and Bocquet 1975b); fresh body weight and ovariole number (David and Bocquet 1975a), chromosome inversion frequencies (Knibb, 1982; Knibb, Oakeshott and Gibson 1981; Stalker 1980), allele frequencies (Oakeshott, Chambers, Gibson and Willcocks 1981; Oakeshott, Gibson, Anderson, Knibb, Anderson and Chambers 1982; Singh, Hickey and David 1982), oviposition rhythmn (Allemand and David 1976) and ethanol tolerance (Cohan and Graf 1985) in *D. melanogaster*; colour pattern polymorphism in the Australian grasshopper *Phaulacridium vittatum* (Dearn 1981); wing pattern variation in the common brown butterfly *Heteronympha merope merope* (Pearse and Murray 1981); life-history traits in the false melon beetle *Atrachya menetriesi* (Ando 1983); and allele frequency in the blue mussel *Mytilus edulis* (Koehn, Milkman and Mitton 1976). Other studies in *D. melanogaster* (Hickey 1979) and *Drosophila pseudoobscura* (Sokoloff 1965) failed to find significant latitudinal clines.

Although many workers have been interested in latitudinal clines, adaptations to altitudinal gradients have also received some attention. Berven (1982a,b, 1987) documented variation in life-history traits between mountain and lowland populations of the woodfrog, *R. sylvatica*. The populations differed genetically in egg size, egg number, and to a lesser extent, age and size at first reproduction. It was hypothesized that selection for larger egg size in the mountains indirectly favours larger adult body size and delayed reproduction of mountain females (Berven 1982a). Reciprocal transplant experiments in the field, and controlled laboratory experiments revealed that most of the observed variation in larval development in *R. sylvatica* between high and low elevation populations could be explained by the effects of temperature induction during ontogeny (Berven 1982b).

Dearn (1977) examined certain life-history characteristics within and between three species of closely related Acridid grasshoppers, *Kosciuscola cognatus*, *Kosciuscola usitatus* and a species belonging to the genus *Praxibulus*, along altitudinal transects in South East Australia. The length and predictability of the summer growing season were considered important in determining the evolution of life-history characteristics along the altitudinal

gradient. However, estimates of the genetic component underlying phenotypic differentiation between adjacent populations were unavailable because of the difficulty in establishing laboratory cultures.

Tanaka and Brookes (1983) attempted to elucidate physiological mechanisms that enable the cricket *Allonemobius fasciatus* to inhabit an altitudinal gradient. Their results failed to provide any conclusive evidence for a genetic difference among populations from different altitudes.

1.3.3 Intrapopulation Comparisons

In addition to interspecific and interpopulational comparisons, life-history variation can be observed at the level of the individual. Differences between individuals of a given population give rise to "differential fitness", a necessary ingredient for evolution by natural selection. However, Stearns (1976) stressed that life-history tactics are only measureable on populations, and not on individuals, for the variance among individual patterns is one of the most important elements of a tactic. He did not intend to circumvent the importance of individual selection but merely to indicate that life-history strategies are observable at the population level. Rather than consider the individual as possessing a particular "tactic", it is more relevant from the evolutionary point of view to consider the individual as being the "temporary custodian" (after Crawford 1984) of particular genes. Depending upon the fitness of the individual it may or may not contribute genes to the following generation.

In conclusion, theoretical arguments can be made either for (Stearns 1976) or against (Stearns 1980) the expectation of intraspecific differences in life-history traits and strategies, with empirical data existing to support both views. The primary difficulty with comparisons made between species or at higher taxonomic levels is, as implied by Brown (1983), that it becomes increasingly difficult to identify the important selective factors responsible for the observed patterns. While comparisons among species or at higher taxonomic levels are valuable in identifying trends and in suggesting forces of natural selection that might operate during the evolution of life-histories, studies at the intraspecific level are necessary to distinguish between real evolutionary alternatives.

1.4 Quantitative Genetics and the Study of Life-Histories

A major contention of life-history theory is that the suite of traits that compose an organisms' life-history are "coadapted" and "designed, by natural selection, to solve particular ecological problems" (Stearns 1976). Evolutionary change in response to natural selection requires that phenotypic variation be heritable, as well as that fitness varies according to the phenotype (Endler 1986). Therefore, meaningful evolutionary interpretations of life-history patterns require that a distinction be made between genotypic variation and environmentally induced variation (Berven 1982a). Phenotypic variation, although necessary for evolutionary change, by itself is not sufficient. Further, phenotypic variation for a particular trait does not necessarily give a guide to the amount or nature of genetic variation, thus theories and concepts based only on the observation of phenotypic variation may be misleading (Barker and Thomas 1987).

A better comprehension of the nature of life-history evolution requires the integration of ecological and genetical disciplines (Etges 1982). Together, the disciplines of ecology and genetics can describe the adaptation of an organism to its environment through evolution by the process of natural selection (Christiansen 1984). While population genetics considers genes and gene frequencies, and quantitative genetics concentrates on variance and covariance, the ecological approach to life-history analysis seeks to understand the selective and other factors that influence the evolution of life-histories through their effects on fitness (Tauber and Tauber 1986a).

The biometrical approach of quantitative genetics is particularly important for the study of life-history characters, because these characters typically show continuous variation due to underlying polygenic determination (Charlesworth 1984; Denno and Dingle 1981; Dingle 1984; Dingle and Hegmann 1982; Etges 1982; Istock 1981, 1982, 1983, 1984; Lande 1982a,b; Lawrence 1984; Mitchell-Olds 1986; Mitchell-Olds and Rutledge 1986; Stearns 1980, 1982a, 1983c,d; Wyngaard 1986b). Polygenes are defined as a "multilocus set where each allele at each locus carries an incremental contribution to the mean and variance of the phenotypic trait in question" (Istock 1983).

The distinction between genes concerned with Mendelian or discrete characters and those concerned with quantitative characters lies in the magnitude of their effects relative to other sources of variation and not on their mode of inheritance (Falconer 1981). However, the multifactorial nature of the inheritance of life-history traits, together with environmental

influences, implies that only rarely can the individual genes influencing the trait be identified (Hill 1984). The theory of gene frequency evolution therefore cannot be directly applied to classical evolutionary problems concerning whole organisms and their phenotypes (Bürger 1986a; Lande 1982b), and often a biometrical genetical investigation is the only practical means of determining the genetical architecture of a continuously varying character (Jinks 1979).

Assuming that variation in life-history characters is caused by many genes of small effect, then quantitative genetic parameters may quantify constraints on the rate and direction of microevolutionary change (Mitchell-Olds 1986). This means that response to selection may be reliably predicted over a few generations, for a particular population in a particular set of environments (Berven and Gill 1983; Istock 1984; Jinks 1979; Mitchell-Olds 1986; Mitchell-Olds and Rutledge 1986; Palmer and Dingle 1986; Schluter and Smith 1986; Stearns 1983c; Travis, Emerson and Blouin 1987). Geographically isolated populations of the same species that have presumably undergone evolution and genetic differentiation in response to their separate environments are expected to display different organizations of their genetic architecture (Wyngaard 1986b). Therefore, a quantitative genetic analysis can also be useful in making inferences regarding the past selective regimes experienced by such populations. However, under many circumstances it is difficult to deduce the past history of selection from the genetic architecture of quantitative traits (see Mitchell-Olds and Rutledge 1986).

Quantitative genetic methods have allowed the effective description and manipulation of polygenic inheritance. These methods are covered clearly and in detail by Bulmer (1980), Falconer (1981), Pirchner (1983) and Becker (1984), so a brief summary will suffice here.

The central assertion of quantitative genetics is that the phenotype of an individual is a unique consequence of its genotype interacting with the sequence of environments that it encounters as a developing organism (Gupta and Lewontin 1982). In order to perform adequate tests of the assumptions or predictions of life-history theory, empirical investigators must perform analyses which allow them to partition the phenotypic variances and covariances of the observed traits into their respective components (Giesel, Murphy and Manlove 1982a). In the case of a single trait the following terms are appropriate:

$$V_P = V_G + V_E + V_{GE}$$

where

V_P = the phenotypic variance of the trait;

V_G = the component attributable to the genetic variance;
V_E = environmentally induced variation; and
V_{GE} = the interaction component between the environment and the genotype in determining the phenotype.

In theory, the genetic variance itself may be further partitioned into additive (V_A), dominance (V_D) and epistatic or interaction (V_I) variance components (Falconer 1981). A further distinction can be made between additive and non-additive components — the former providing the basis for selection response. Even though a knowledge of the relative magnitudes of these components gives some understanding as to the nature of quantitative genetic variation, it must be recognised that it is only a statistical description of variation and not one based on a direct analysis of gene action (Barker and Thomas 1987).

In a biometrical analysis of covariance, a similar partitioning of covariance between different traits into genetic, environment and genotype-environment interaction components can be made.

Most quantitative genetic analyses of variation and covariation, and the derivation of associated statistical variables, are based on several important assumptions, most of which can be ensured by proper experimental design (Barker and Thomas 1987; Mitchell-Olds and Rutledge 1986). Some of these assumptions have been, according to Barker and Thomas (1987), "stated explicitly in the development of the theory, some are believed to be true and some are known not to be true (at least sometimes)". The usual restrictive assumptions include:

a) the genes affecting a particular trait are inherited as normal Mendelian genes;

b) the traits are determined by the joint effects of the environment and a large number of unlinked, non-interacting loci (no epistasis), with approximately equal, additive and small effects on the individuals phenotype (polygenic inheritance);

c) the population is randomly mating, with Hardy-Weinberg equilibrium genotype frequencies — no inbreeding, selection, migration or assortative mating;

d) no correlation between genotype and environment — genotypes are randomly allocated throughout the entire experiment; and

e) there is no genotype-environment interaction.

Other implicit assumptions commonly include:

1) the genetic variation is due to structural genes: and

2) no competition or cooperation among individuals within the population, or between these individuals and members of other species, beyond random expectation.

Probably, the most important assumption in any quantitative genetic analysis of natural populations is that the individuals used are a random sample of the reference population (Mitchell-Olds and Rutledge 1986). Before undertaking any quantitative genetic analysis, and particularly before interpreting the results, violations of these assumptions should be recognised and their possible effects on interpretation considered.

1.4.1 Quantitative Genetic Variables

Theory argues that both the direction and rate of response to selection will be determined in part by the amounts of additive genetic variance and covariance for the different life-history characters. The two important variables from classical quantitative genetics which bear on the evolutionary dynamics of variation and covariation in polygenic traits are the heritability of a trait, and the genetic correlation between traits (Falconer 1981).

1.4.1.1 Heritability

The heritability of a trait, in the narrow sense, is defined as the ratio of the additive genetic to the phenotypic variance:

$$h^2 = V_A/V_P$$

It expresses the proportion of the phenotypic variance that is attributable to the average effects of genes, and this is what determines the degree of resemblance between relatives. The most important function of the heritability is its predictive role, in expressing the reliability of the phenotypic value as a guide to breeding value (Falconer 1981).

1.4.1.2 Genetic Correlation

If a life-history "tactic" is an assemblage of characters which function as a coadapted unit (Stearns 1976), then the ability of the life-history to respond to the environment will also depend upon the nature of the relationships among the different traits. Since natural

selection acts simultaneously on many life-history characters (which are those associated with fitness), they cannot evolve independently because of genetic correlations between them caused by pleiotropy and linkage disequilibrium (Lande 1982a,b). The adaptive response to selection on the entire life-history may thus involve compromises or "trade-offs" between characters that are genetically correlated (Giesel et al. 1982a; Istock 1984; Lande 1982a,b).

A genetic correlation is defined as the genetic covariance between two characters standardized by the genetic variances:

$$r_G = \frac{cov_{G(XY)}}{\sqrt{(var_{G(X)})(var_{G(Y)})}}$$

where $cov_{G(XY)}$ is the genetic covariance of the additive deviations of characters X and Y, and $var_{G(X)}$ and $var_{G(Y)}$ are the genetic variances for each of the characters (Via 1984b).

One of the limitations in using heritabilities and genetic correlations to predict response to selection is that they are statistical descriptions of variance and covariance specific to particular populations in particular environments (Falconer 1981). Providing heritability and genetic correlation estimates are not extrapolated beyond these circumstances, they can be very informative in predicting future response to selection and making inferences about past selection. The often neglected component in many quantitative genetic analyses of phenotypic variation is the component of variance due to the interaction between the genotype and the environment (V_{GE}).

1.4.1.3 Genotype-Environment Interaction

A species is rarely confined to a single uniform environment, but usually inhabits a range of environments differing over time and/or space. For a species to persist in a heterogeneous environment the individuals of that species must be either phenotypically flexible or genetically variable. Phenotypic flexibility of an individual is the extent to which it can grow and reproduce in a range of environments either by varying its phenotype or by maintaining a constant phenotype (homeostasis and stability) (see Scheiner and Goodnight 1984).

The capacity of a single genotype to produce a range of environment-dependent phenotypes is the phenotypic plasticity of an individual (Bradshaw 1965). When discussing phenotypic plasticity it is useful to distinguish between two main types. One type is the

all-or-nothing response that results from an environmentally cued switch between two alternative developmental programs. This type of response has been called "autoregulative development" (Schmalhausen 1949) or "developmental conversion" (Smith-Gill 1983). The second type of phenotypic response is more continuous, and may be adaptive or may result from physiological constraints on development. Responses of this kind have been called "dependent morphogenesis" (Schmalhausen 1949) or "phenotypic modulation" (Smith-Gill 1983), and are the types of plasticity dealt with in this thesis.

The profile of phenotypes that will be produced by a genotype over an array of environments is the "norm of reaction" of a genotype (Schmalhausen 1949). In order to determine experimentally the reaction norm of a genotype with respect to a particular phenotypic character and a set of environments, it must be possible to replicate the genotype, characterizing its phenotype in each environment. Since this leads to large experiments, little information exists on reaction norms of genotypes taken from natural populations (Gupta and Lewontin 1982).

Phenotypic plasticity is a necessary pre-requisite for the detection of any genotype-environment interaction (Via and Lande 1985, 1987). If the phenotypic plasticity displayed by genotypes within a population varies among genotypes, then there is the potential for genotype-environment interaction. Further, within-population genotype-environment interaction (the primary cause of which is mutation) is necessary for the evolution of phenotypic plasticity.

Genotype-environment interaction is defined by Pani and Lasley (1972) as the "differential response of a genotype in different environments". Since the phenotype of an individual is completely determined by its genotype and environment, the statistical detection of an interaction means that the effects of genotype and environment are not independent (Via 1984a). Independence of effects simply means that the contribution of a particular genotype to the formation of the phenotype does not depend on the environment, and conversely, that a particular environment makes the same contribution to the phenotype, irrespective of the genotype on which it acts (Falconer 1981; Gregorius and Namkoong 1986).

The interaction can be of several forms. A specific environmental difference may have a greater effect on some genotypes than on others (scaling), or there may be a change in the order of merit (ranking) of a series of genotypes when measured under different environments (Falconer 1981). Genotype-environment interaction is often treated in quantitative genetics literature merely as a scaling problem (Gimelfarb 1986a). It is frequently assumed that a

scale transformation of a quantitative character can be found such that the genotypic and environmental effects become additive, or near additive, on the transformed scale. Although a transformation reducing the interaction can be found in some, if not many instances, this is not always the case, and genotype-environment interaction may represent a real biological phenomenon.

The consequences of a change in ranking of genotypes measured in different environments is more important from an evolutionary perspective. In such a case, if natural selection were to proceed separately in several environments, the selected populations would be expected to evolve different genetic constitutions (Via 1984a,b). Not only can the course of phenotypic evolution vary among populations inhabiting different environments, but environmental dependence of the variance-covariance matrix makes it much more difficult to obtain realistic estimates of these matrices for natural populations (Via 1984b).

Even when estimates of heritabilities and genetic correlations are obtained under uniform laboratory conditions, such estimates must be kept in context. Unless genotype-environment interaction is non-existent, extrapolation to environments different to those in which estimates were made, remains risky and undesirable. Further, for organisms occupying heterogeneous environments, only those studies which address the possibility of genotype-environment interaction will produce realistic estimates of the genetic parameters required for microevolutionary theory (Via 1984a).

The biological importance of any genotype-environment interaction can be better appreciated by expressing the interaction in terms of a genetic correlation between the same trait measured in different environments, rather than from the qualitative estimates of significance obtained from analyses of variance (Murthy and Taneja 1982; Robertson 1959b). The correlation format is also more useful mathematically, because these estimates of genotype-environment interaction can be readily incorporated into evolutionary theory (Via and Lande 1985).

Falconer (1952) first noted that a character expressed in two environments could be regarded as two different characters which are genetically correlated. On this basis, the genetic correlation between the trait measured in the two environments indicates whether or not genotype-environment interaction is present, and the degree to which the phenotypes have the same genetic basis. A genetic correlation of nearly one implies that genotype-environment interaction variance is negligible, and that the same alleles or sets of alleles influence the "character states" (after Via and Lande 1985) in the same way in the two

environments. In contrast, a genetic correlation across environments less than unity would imply the presence of genotype-environment interaction and indicate that the phenotypes in each environment are influenced either by some different alleles or differently by the same alleles (Via and Lande 1985, 1987). Robertson (1959b) suggested that a correlation around 0.8, or less, would be of biological importance.

In the analysis of genotype-environment interaction, any given individual experiences only one environment. To estimate the genetic correlation between character states expressed in different environments, replicated genotypes or family members are allowed to develop in the different environments. Because measurements of the different character states are not made on the same individuals the usual statistical procedures for calculating the genetic correlation are not applicable, and alternative methods must be used.

Alternative methods include the standard family mean correlation and the correlation based on arbitrary pairing of family members (Via 1984b), or the re-expression of the genotype-environment interaction variance component from the analysis of variance as a genetic covariance between character states expressed in different environments (Dickerson 1962; Robertson 1959b; Yamada 1962). Fernando, Knights and Gianola (1984) have indicated that if the data are unbalanced, the mixed model method of Yamada (1962) gives biased estimates of genetic covariances unless the traits expressed in the different environments have identical genetic and residual variances. This criticism is yet to be resolved (Yamada pers. comm.). Schaeffer, Wilton and Thompson (1978) presented a general solution to the problem of estimating correlations between traits observed on different experimental units. The procedure, which allows for different linear models in the different environments, consists of maximizing the likelihood of a set of error contrasts. However, computations are formidable and require iteration, and convergence may be slow or may not occur in some instances.

Obviously, there are a variety of ways to detect and quantify the importance of genotype-environment interactions. Not all methods that express the interaction as a genetic correlation have been thoroughly evaluated in terms of their accuracy and applicability under practical circumstances (Mathur and Horst 1985). Presently it seems that a combination of methods, both qualitative and quantitative, would be beneficial in demonstrating the importance of this phenomenon to the evolution of life-histories in natural populations.

To comprehensively study life-history evolution in a particular species, it would seem logical to at least:

1) use natural populations:

2) make intraspecific comparisons between natural populations:

3) separate, by a quantitative genetic analysis, the genotypic and environmental effects which determine the phenotypes of individuals within populations, and further

   a) estimate heritabilities for individual traits:

   b) estimate genetic correlations between the different traits which make up a life-history; and

   c) allow for the possibility of genotype-environment interaction.

The term "natural" population is used here to describe a population studied in the wild, or if sampled from its natural habitat and introduced to the laboratory, one which will have undergone minimal adaptation to the new environment, effectively therefore, retaining the same genetic constitution.

The magnitude of any empirical investigation attempting to satisfy all of the above criteria and still obtain reliable estimates, may well be, and often is, experimentally unmanageable. Although one should at least consider all of the above criteria prior to an experiment aimed at elucidating aspects of life-history evolution in a particular species, studies which do not cover all aspects remain informative, and are still needed.

1.5 Attempts to Explain Patterns in Life-History Evolution

The goal of evolutionary ecology is to build theories that make successful predictions of the evolutionary changes in organisms in response to specified changes in their environment (Stearns 1982c). If the life-history "strategy" is composed of a set of interrelated traits "designed" by natural selection to solve particular ecological problems (Stearns 1976), it is more than likely that each individual, each population and each species will have its own unique life-history strategy (Begon and Mortimer 1986). In order to study the life-history phenomenon it has been necessary to attempt to discern patterns from the multiplicity of available life-histories observed in nature. In any attempt to simplify such complexity, two logical questions arise:

a) Are there discernible patterns among the traits that make up a life-history strategy whereby certain traits are generally associated with certain other traits? and

b) Are there any obvious patterns in which particular types of life-history strategy are associated with particular types of habitat?

In response to the first question, two basic types of life-history are sometimes recognizable in nature, and their easy identification stimulated the initial interest in life-history evolution (MacArthur and Wilson 1967; Pianka 1970). The two patterns are: early maturation, many small young, high reproductive effort and short life, as compared with late maturation, a few large offspring, small reproductive effort and a long life. These patterns were considered to be the result of ecological selection pressures (natural selection), and their identification gave the first impetus to the development of the theory of reproductive cost. Attempts to explain these patterns have largely fallen into three categories — "r- and K-selection", "bet-hedging" and what will be referred to as the "modern theory". The first two are purely ecological theories, whereas the latter can be extended to incorporate a genetic basis.

MacArthur and Wilson (1967) introduced the terms r- and K-selection and identified the density of a species with respect to its resources as the selective pressure capable of explaining these two fundamental groupings of life-history traits. Originally r-selection meant selection for high population growth in uncrowded populations and K-selection referred to selection for competitive ability in crowded populations.

However, Parry (1981) has pointed out that the definitions of r- and K-selection have been developed and reinterpreted in many different ways in the literature, and, to add to the confusion, authors often use the different meanings interchangeably. As Brookfield (1986) notes, it is not always clear whether r- and K-selection are just terms for species with high reproductive rates and low reproductive rates, or whether some evolutionary change is being inferred.

Parry (1981) recognized three extensions to the original concept of MacArthur and Wilson (1967) corresponding to contrasts of density-dependent versus density-independent regulation, ephemeral versus stable habitats, and large versus small reproductive efforts. The broadening of the concept has only led to confusion within the literature, and Parry (1981) recommends abandoning all the definitions except that of MacArthur and Wilson (1967).

May and Rubenstein (1985) consider the dichotomy of r-selection versus K-selection as "a gross oversimplification, which deliberately polarizes what is, in fact, a complex continuum". In natural populations selective forces are usually diverse and attempts to explain

life-histories as outcomes of a single selective pressure are inadequate (Barclay and Gregory 1981; Begon and Mortimer 1986; Boulétreau-Merle, Allemand, Cohet and David 1982; Deshmukh 1986; May and Rubenstein 1985; Parry 1981; Price 1984; Stearns 1984; Tallamy and Denno 1981; Tinkle and Hadley 1975; Wilbur et al. 1974). Another difficulty with the r- and K-selection concept is that it ignores the existence of age structure in populations and the ecological differences that may exist among members of different ages within the same population (Barclay and Gregory 1981).

An alternative hypothesis, developed from the theoretical papers of Istock (1967), Murphy (1968) and Schaffer (1974b) deals specifically with the consequences of fluctuating mortality schedules. Stearns (1976) discusses the relevant predictions under the heading of "bet-hedging". With unpredictable adult mortality, selection in fluctuating or stable environments favours the combinations of traits predicted by r- and K-selection respectively. However, when juvenile mortality fluctuates due to unpredictable environmental conditions, selection favours organisms with the same combination of traits predicted under K-selection. If fluctuations in juvenile mortality become more predictable as a result of stable environments, organisms that mature earlier, are iteroparous, have larger reproductive efforts, shorter lives, more young per brood and fewer broods, are favoured. Therefore, when juvenile mortality fluctuates, the bet-hedging theorem predicts combinations of traits, more or less, opposite to those predicted by r- and K-selection.

Stearns (1977) after examining the available literature on life-history diversity concluded that neither the deterministic model of r- and K-selection nor the stochastic model of bet-hedging were empirically sufficient — predictions were not consistent with much of the evidence. It seems that the bet-hedging theorem, or any ecological theory which relies on demographic parameters as the sole evolutionary pressure on life-histories, is limited in its application.

Accepting the inadequacies of "r- and K-selection" and "bet-hedging", it becomes desirable to find alternative theories to predict how life-histories might evolve in a particular situation. Stearns (1983b) considers age-specific mortality and fecundity rates, and the cost of reproduction to be important elements in such a theory. This theory, which shall be called the "modern theory", needs to consider the possibility of multiple ecological forces operating on a particular population at any given time. Reproductive cost, a component of the modern life-history theory is discussed in the next section. The importance of distinguishing between genotypic and environmentally induced variation remains fundamental.

1.6 The Cost of Reproduction

The one assumption which is common to most theories of life-history evolution is the concept of "trade-offs" (Wu 1981). A trade-off in life-history theory means that an improvement in one fitness related character is associated with a decrement in some other fitness related character (Reznick 1985). One particular trade-off of fundamental importance to life-history evolution is the cost of reproduction (see Bell 1980, 1984a,b, 1986; Reznick 1985; Reznick, Perry and Travis 1986). Life-history theory predicts the optimal age-specific allocation of resources to growth, maintenance and reproduction (Askenmo 1979; Gadgil and Bossert 1970; Reznick et al. 1986). The strategy adopted by an organism is therefore a compromise allocation of energy to the various aspects of its life-history, each of which contributes to total fitness (Begon and Mortimer 1986).

"Reproductive effort", a concept used in much of the literature (Askenmo 1979; Barber and Blake 1986; Charlesworth and León 1976; Gadgil and Bossert 1970; Gremare and Olive 1986; Haukioja and Hakala 1978; Reekie and Bazzaz 1987a,b,c; Samson and Werk 1986; Schaffer 1974a,b; Williams 1966a,b), is the proportion of available resources of time and/or energy which are committed to reproduction rather than to maintenance or growth. Bell (1980) considers this concept redundant and recommends that it be discarded, because it is defined in terms of units (calories, seconds) which are, in themselves, irrelevant to the evolution of life-histories. Further, the direct measurement of reproductive effort is technically difficult and laborious, and the results meaningless unless they can be related to effects on fitness.

As a replacement to reproductive effort, Bell (1980) recommends using, for both theoretical and empirical work, the related concept of "reproductive costs" for interpreting age-specific schedules of reproduction. The "reproductive cost" hypothesis states that any increment in present reproduction is associated with a decrement in the expectation of future reproduction (Bell 1980, 1984a,b). Such a decrement in the expectation of future reproduction may result from either a reduction in future survival and/or a reduction in future fecundity.

Reproductive costs may be of two kinds (Gremare and Olive 1986). Firstly, and independently of the environment, a decrease in future survival and/or fecundity may result from a diversion of energy from somatic investment into reproduction. Following this diversion of energy, an individual with a limited quantity of available resources, may no longer be able

to satisfy its energy requirements for growth and maintenance. Secondly, a cost to future reproduction may be incurred through the organism's interaction with its environment. By diverting more energy into reproduction an organism becomes more vulnerable to its habitat. Bell's (1980, 1984a,b) definition of reproductive cost embraces both kinds, including those costs whose causation is not so easily related to a decrement in energy allocation. However, some authors (see Bell 1984a,b, 1986; Reznick 1985; Reznick et al. 1986) have, on the basis of available empirical evidence, questioned the universal applicability of the concept of reproductive cost.

The theory of reproductive cost implies a negative correlation between the present reproduction of individuals and their expected future reproduction when they experience the same conditions of life (Bell 1984b). Therefore a common approach for evaluating the cost hypothesis involves measuring the correlations between the appropriate life-history variables. For example, if there really is a cost associated with increased reproductive investment early in life, then one would expect this variable to be negatively correlated with survival or with the amount of future reproduction (Reznick et al. 1986). However, a negative correlation between reproduction and growth is only suggestive of decreased future fecundity and must not be used as evidence for a reproductive cost unless a decrease in growth can be shown to have a direct causal effect on future mortality and or fecundity (Bell 1984a).

Life-history theory predicts how the life-history will evolve in a particular population under specified environmental circumstances (Stearns 1976). Because these are evolutionary theories which predict evolutionary response, it is necessary to establish a genetic basis to the correlation before it can be used to infer that a reproductive cost has evolutionary consequences (Reznick 1985; Reznick et al. 1986).

The importance of the cost of reproduction in life-history theory has induced a large number of investigators to seek empirical evidence. It is not the purpose of this thesis to review the many studies which are comprehensively dealt with by Bell (1980, 1984a,b), Reznick (1985), and Reznick et al. (1986). However, it is important to identify the methods available for estimating reproductive costs and to establish whether or not they can be considered a universal phenomenon.

Reznick (1985) classified empirical studies into four categories, and then examined which experimental designs were more appropriate for measuring reproductive costs. The four methods for measuring the cost of reproduction include:

a) studies which measure phenotypic correlations:

b) studies which directly manipulate some aspect of the life-history (usually reproduction) and/or some environmental variable which affects reproduction;

c) studies which measure genetic correlations; and

d) studies which examine correlated responses to selection.

Studies estimating phenotypic correlations generally measure the correlation or association between some index of reproductive effort and a potential cost to the parents in terms of survival, growth, or future fecundity (Reznick 1985). Since phenotypic correlation estimates include the combined effects of genotype and environment, they do not necessarily reflect the underlying genetic correlations (Falconer 1981; Rose and Charlesworth 1981a). Therefore, phenotypic correlations as a method of evaluating the existence of reproductive costs and assessing their importance to life-history evolution, are unsatisfactory (Reznick 1985).

The second method, experimental manipulations, involves the direct alteration or manipulation of some aspect of the life-history, such as whether or not an individual is able to reproduce. Responses in other traits such as adult survival or adult growth are then compared between reproductive and non-reproductive individuals. Alternatively, some aspect of the environment such as food availability can be manipulated rather than the life-history itself, and correlations between life-history variables measured. These studies therefore assess the ability of a genotype to adjust phenotypically to changed environmental circumstances. Since phenotypic plasticity does not necessarily reflect underlying genetic correlations (Reznick 1985), experimental manipulations, as a method, are also inadequate for measuring the cost of reproduction. However, both experimental manipulations and phenotypic correlations are of some value insofar as they demonstrate how costs might be manifested in real organisms and what their magnitudes might be (Reznick 1985).

The more suitable techniques for measuring costs of reproduction include the estimation of genetic correlations and observing correlated responses to selection (Bell 1986; Reznick 1985). Both of these methods can establish whether or not reproductive costs have a genetic basis.

The estimation of genetic correlations, as in any quantitative genetic study, is subject to limitations. The estimates are particular to a given population in a given environment (Falconer 1981). Genotype-environment interactions are potentially important in determining both the magnitude and sign of genetic correlations between different life-history traits

(Giesel 1986b; Giesel et al. 1982a,b; Murphy, Giesel and Manlove 1983). Since genetic correlations are highly dependent on gene frequency, Reznick (1985) suggests that measurements on populations where gene frequencies are changing can yield highly variable results. While Reznick's (1985) criticism of the usefulness of the correlation matrix in making long-term predictions may be correct, it is irrelevant to the question at hand, the detection of reproductive costs.

Both the estimation of genetic correlations and selection experiments incompletely assess the costs of reproduction because they only consider interactions within individuals. They do not detect the type of costs attributed to interactions between individuals and external environmental factors (Haukioja and Hakala 1978; Tuomi, Hakala and Haukioja 1983). Further, most empirical studies consider only the presence or absence of costs, and not the nature of the relationship which is important for predicting the direction of life-history evolution (Bell 1986; Reznick 1985).

There are several explanations why reproductive costs may not always be present. For example, Tuomi et al. (1983) suggest that reproducing individuals may be able to reduce or offset the various costs of reproduction by either increasing their resource intake and ingestion during reproductive periods relative to non-reproducing individuals, or by utilizing previously deposited somatic reserves for reproduction.

In conclusion, the universal applicability of the cost of reproduction has not been established, especially for natural populations. Empirical studies on natural populations from a diversity of species must be done with measurements of genetic correlations or correlated responses to selection. Until such evidence becomes available, the importance of reproductive costs to the evolution of life-histories remains speculative.

1.7 The Maintenance of Quantitative Genetic Variation

Most traditional inferences about the quantitative genetics of fitness traits, and therefore, life-history traits, in natural populations are based on Fisher's (1930, 1958) "Fundamental Theorem of Natural Selection". This theorem has been so important because it reconciled Darwinian evolution with genetics by linking the evolutionary improvement of the species with individual fitness and the genetic variation of fitness within species (Christiansen 1984).

Fisher (1930) postulated that under certain idealized conditions "the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time".

A corollary to the theorem proposes that as a population under natural selection reaches equilibrium, fitness differences approach zero, and consequently additive genetic variance in fitness is soon depleted (see Etges 1982; Gustafsson 1986; Istock 1978, 1983; Mitchell-Olds 1986; Rose 1984b). In addition, there is also a so-called secondary theorem which states that the expected change in a metric character produced by any selection process is equal to the genetic covariance between the character concerned and relative fitness (Robertson 1966, 1980).

If there is long-term directional selection for fitness and its major components in outbred populations, then life-history traits (reproduction and survival) which are strongly correlated with fitness, are expected to display low levels of additive genetic variance, and therefore, low heritabilities. However, there seems to be universal agreement that abundant heritable variation exists for at least some life-history traits in many laboratory and natural populations for many species (Allan 1984; Barker and Thomas 1987; Cade 1984; Charlesworth 1984; Clark 1987; Dawson and Riddle 1983; Ennos 1983; Falconer 1981; Giesel et al. 1982a,b; Gilbert 1984; Giles and Edwards 1983; Gustafsson 1986; Hegmann and Dingle 1982; Istock 1981, 1983, 1984; Istock, Zisfein and Vavra 1976; Lewontin 1986; Mitchell-Olds 1986; Mitchell-Olds and Rutledge 1986; Mousseau and Roff 1987; Murphy et al. 1983; Riddle, Dawson and Zirkle 1986; Roff and Mousseau 1987; Rose 1984b; Rose and Charlesworth 1981a,b; Schwaegerle et al. 1986; Tauber and Tauber 1986b; Tauber et al. 1986; Venable 1984; Wyngaard 1986b).

These findings of significant amounts of additive genetic variance in life-history traits have instigated a great deal of discussion about the validity and generality of Fisher's Fundamental Theorem (Rose 1984b). For example, even though Fisher's (1930, 1941, 1958) explanations of his theorem were "afflicted by a truly astonishing number of obscurities, infelicities of expression, typographical errors, omissions of crucial explanations, and contradictions", Price (1972) still considered it to be mathematically correct, but less important than originally thought. While clearly lacking the power and generality originally intended, the quantification of the relation between variance and response to natural selection has still been important in predicting rates of evolutionary change (Hill 1984).

The expectation of a lack of genetic variation for life-history traits presumably reflects an uncritical application of the theorem. In contrast to Fisher's idealized conditions underlying the theorem, most natural populations occupy spatially and or temporally heterogeneous

environments, and display varying amounts of mutation. Further, heritabilities for individual fitness components may not necessarily reflect the amount of additive genetic variance for the composite trait, total fitness, to which the theorem refers (Rose and Charlesworth 1981b). Obviously, the more components and their interactions considered, the closer a composite measure should be.

Most natural populations are probably close to equilibrium, and therefore high values of additive genetic variance may persist only for characters which are unrelated to fitness (Falconer 1981). Empirical studies of domestic animals and laboratory populations of *Drosophila* show lower heritabilities for traits that probably exert some influence on fitness (Falconer 1981). Gustafsson (1986) in studying the heritabilities of morphological characters, components of fitness, and lifetime reproductive success in a natural population of the collared flycatcher *Ficedula albicollis*, reported an inverse relation between the heritability of a trait and its influence on fitness. As predicted the heritability of lifetime reproductive success was close to zero.

Roff and Mousseau (1987) examined patterns of heritability in the genus *Drosophila*. Despite biases and large confidence intervals for many of the estimates, the data strongly suggested that life-history traits do have consistently lower heritabilities than morphological traits, but possibly not behavioural traits. Mousseau and Roff (1987) examined patterns of heritability for behavioural, life-history, morphological and physiological traits in natural populations from 75 different species, excluding the genus *Drosophila*. Their findings support the notion that traits closely associated with fitness generally show lower heritabilities than those that are more distantly related. The ranking of trait types was consistent with that for the genus *Drosophila* (Roff and Mousseau 1987). Further, the magnitude of the average heritabilities of these traits suggested that significant genetic variance is maintained within most natural populations, even for traits closely associated with fitness. Thus, the relative amounts of additive genetic variance appear to be in keeping with the predictions of the Fundamental Theorem, at least sometimes.

If heritabilities are intrinsically low for fitness characters, it will be more difficult to detect very small amounts of additive polygenic variation, amounts which appear to be consistent with an interpretation of no heritability. However, as Etges (1982) notes, even a small amount of additive genetic variance approaching statistical significance may still play an important role in the evolution of life-histories.

What is responsible for the maintenance of significant amounts of additive genetic variation in life-history traits of natural populations?

The simplest explanation is that it is generated by mutation. The mutation-selection balance hypothesis is particularly appealing because it involves an equilibrium between stabilizing phenotypic selection, which tends to eliminate additive genetic variance, and mutation, which continually reintroduces it (Turelli 1985). A number of theoretical models have been developed to incorporate mutations as a source of variation (Barton 1986; Barton and Turelli 1987; Bulmer 1980; Bürger 1986b; Kimura 1965; Lande 1975; Narain and Chakraborty 1987; Turelli 1984, 1985). It seems these models have been unable to conclusively establish the extent to which mutation is responsible for the levels of additive genetic variation empirically observed. Empirical estimates of actual mutation rates, gametic mutation rates, mutability of loci, number of loci affecting a trait, and the strength of stabilizing selection acting on a trait are needed to establish the validity of the various models (Clegg and Epperson 1985). It is likely that mutation does play some role, but probably not a sufficient one. Therefore, there is little reason for concentrating exclusively on mutation-selection equilibrium as a mechanism for maintaining polygenic variation (Barton and Turelli 1987).

Another mechanism which, theoretically, can contribute to the maintenance of significant additive genetic variance for fitness related characters, and therefore life-history traits, is "antagonistic pleiotropy" (see Barker and Thomas 1987; Gimelfarb 1986b; Roff and Mousseau 1987; Rose 1982, 1983a,b, 1984b, 1985a; Rose et al. 1987; Service and Rose 1985). Assuming the life-history "tactic" of an organism is made up of an assemblage of characters which function as a coadapted unit (Stearns 1976), then separate life-history traits may have no independent existence in nature (Tuomi et al. 1983) — they are always dependent upon the structural and functional organization of the individual organism.

The existence of high additive genetic variance in fitness components is not inconsistent with the expectation that fitness itself should display low or zero genetic variance in an equilibrium population (Rose 1982) provided there is "antagonistic pleiotropy", or genetic "trade-offs", between the individual fitness components. In large populations, after many generations of natural selection, negative genetic correlations are expected between fitness components, since loci with positively correlated effects will become fixed for the advantageous alleles, whereas loci with antagonistic effects will remain segregating (Falconer 1981).

For the theory of maintenance of genetic variation by antagonistic pleiotropy to hold,

empirical studies are expected to disclose abundant additive genetic variability, associated with negative genetic correlations between genetically variable characters (Rose et al. 1987). Further, these negative correlations are assumed to be due to pleiotropy and not linkage disequilibrium (Rose 1984b).

Empirical studies have reported negative genetic correlations between life-history traits in *D. melanogaster* (Luckinbill et al. 1984; Rose 1984c; Rose and Charlesworth 1981a,b; Rose et al. 1987; Service and Rose 1985; Simmons, Preston and Engels 1980). In contrast, generally positive, or approximately zero, rather than negative correlations have been found in *D. melanogaster* (Giesel 1979, 1986b; Giesel et al. 1982a,b; Giesel and Zettler 1980), *D. simulans* (Murphy et al. 1983), species of freshwater invertebrates (Bell 1984a,b) and the partially selfpollinating herbaceous annual *I. capensis* (Mitchell-Olds 1986). Obviously, the empirical data disagree on the universality of antagonistic pleiotropy, and therefore its generality as a means of maintaining genetic variation in life-history traits. Such a discrepancy in the empirical literature may be due either to a true difference in the genetic correlations, or due to differences in the experimental protocols with some generating spurious correlations (Clark 1987).

Rose (1984a) noted that there is an apparent contradiction between the results found from different laboratories, all working on the same problem of fitness-component covariation within the same species, *D. melanogaster*. The degree of inbreeding in the experimental material has been implicated in generating positive correlations by Rose (1984a), Rose and Charlesworth (1981a) and Mackay (1985). The studies of Giesel et al. (1982a,b) were potentially faulted by partial inbreeding of the *D. melanogaster* experimental stocks which could have, as Rose (1984a) suggested, led to homozygous accumulation of deleterious genes and to different levels of homozygosity for different genes among stock lines, thus producing the observed positive genetic correlations. In response to these criticisms, Giesel (1986b) re-examined the additive genetic correlation structures of early- and late-life traits in a wild, outbred population of *D. melanogaster* under two different environments defined by photoperiod. The additive genetic correlations, when significant, were similarly positive, particularly when flies were reared under putatively optimal conditions. Occasional negative correlations were found between early- and late-life traits, but only when flies had been reared under stressful conditions. However, this study, like many other quantitative genetic analyses, was afflicted by the problem of small sample sizes.

Service and Rose (1985) argue that another aspect of experimental design, namely novel

environmental effects, can also produce artifactual correlations that will tend to be positive. They found a significant reduction in the strength of the negative additive genetic correlation between early-life fecundity and starvation resistance in a *D. melanogaster* population when measured in a novel environment.

The empirical evidence, at least for *D. melanogaster*, suggests that, while the antagonistic pleiotropy hypothesis is attractive, more quantitative genetic studies, appropriately designed and free from the biases discussed by Mitchell-Olds (1986), Mitchell-Olds and Rutledge (1986) and Barker and Thomas (1987), are needed to test its generality. Further, it would be desirable to study natural populations from other species, such as *Tribolium*, where the environment used in laboratory culturing more closely approximates the conditions of the natural habitat.

Most populations of a given species rarely experience uniform conditions in nature, but rather occupy spatially and/or temporally heterogeneous environments. This, along with the ability of different geographic populations to adapt to their particular environmental circumstances, has linked environmental heterogeneity not only with slowing the erosion of additive genetic variance in fitness traits, but also with its maintenance within populations (see Barker and Thomas 1987; Bulmer 1980; Dawson and Riddle 1983; Endler 1986; Ennos 1983; Giesel 1986a; Giesel et al. 1982a,b; Istock 1983; Mackay 1981; Mitchell-Olds 1986; Mitchell-Olds and Rutledge 1986; Murphy et al. 1983; Riddle et al. 1986; Rose 1983a; Rose et al. 1987; Tauber and Tauber 1986a; Via and Lande 1985, 1987).

If genetic variation is to be maintained by fluctuating selection pressures in heterogeneous environments, then different genotypes must have higher fitness in different environmental states (Dawson and Riddle 1983; Riddle et al. 1986). Therefore, a population taken from a heterogeneous environment and isolated in one environmental state should, under the influence of natural selection, evolve a gene pool more adapted to that particular environment. Also, populations isolated in separate states of the heterogeneous environment would be expected to evolve different genetic constitutions. Furthermore, these isolated populations would be expected to contain less within-population additive genetic variance for fitness traits than the original population occupying the heterogeneous environment.

Genotype-environment interactions are expected if environmental heterogeneity is responsible for maintaining a significant component of genetic variation for life-history traits in natural populations, because of the assumption that different genotypes have the highest

phenotypic values in different environmental states. Examples of genotype-environment interaction have been documented in *D. melanogaster* (Giesel 1986b; Giesel et al. 1982a,b), *Eurytemora herdmani* (McLaren 1976), *O. fasciatus* (Baldwin and Dingle 1986; Groeters and Dingle 1987), *M. edax* (Wyngaard 1986a), *L. sativae* (Via 1984a,b), and *D. simulans* (Giesel 1986a; Murphy et al. 1983).

Empirical objections to the hypothesis that heterogeneous environments can maintain genetic variation have been raised by Rose et al. (1987). Firstly, long established laboratory populations maintained under constant laboratory conditions can maintain high equilibrium levels of genetic variance in life-history characters (Rose 1984b; Rose and Charlesworth 1981a,b). Secondly, the expected pattern of dependence of quantitative genetic variability on environmental variation was not found in a study specifically addressing the problem (Dawson and Riddle 1983; Riddle et al. 1986). A long-term "natural" selection experiment with *T. castaneum* and *T. confusum* tested for the ability of synthetic populations to adapt to a series of flour diets made from cereal grains that these beetles commonly invade in nature. Although significant variation and genotype-environment interactions were found for a variety of growth traits and fitness components, the results did not support the hypothesis that genetic variation was maintained by diversifying selection in a heterogeneous environment. There were a number of possible reasons given for the unexpected results (Dawson and Riddle 1983; Riddle et al. 1986). One plausible explanation suggested that pleiotropic effects of genes on different fitness characters were important in determining net fitness values of polygenes, and therefore the maintenance of genetic variation.

Verdonck (1987) investigated the ability of experimental *D. melanogaster* populations to adapt to environmental heterogeneity. For 29 generations, *D. melanogaster* populations were offered one favourable (standard) and one sub-optimal (salt-supplemented) medium, either singly or simultaneously. If environmental heterogeneity acts to maintain genetic variability, then the mixed resource populations were expected to be genetically more variable, with a greater ability to respond to selection than the single resource populations. A significantly greater response to selection for variability in the mixed populations as compared with either of the single resource populations was found in one experiment, but not in the other. These findings offer some, but inconclusive evidence in favour of the hypothesis that environmental heterogeneity promotes the maintenance of genetic variation in populations.

It would be unreasonable at this stage, with so little empirical evidence, and considering the widespread occurrence of genotype-environment interactions, to discount the importance

of environmental heterogeneity in maintaining additive genetic variation for life-history traits of natural populations.

Some other factors which have been implicated in maintaining genetic variation are random genetic drift (Endler 1986; Lande 1976), gene flow (Endler 1977), frequency-dependent and density-dependent selection (Barker and Thomas 1987; Bradley 1982; Hedrick and Murray 1984), linkage disequilibrium (Cade 1984), and heterozygote advantage (Bradley 1982; Ennos 1983; Mackay 1981). The importance of these factors, along with mutation rate, antagonistic pleiotropy and heterogeneous environments in maintaining additive genetic variation for life-history traits of natural populations is yet to be determined. However, in any given population, it is likely that a combination of factors is involved.

Chapter 2

Background to the Experiments

2.1 Experimental Rationale

In the review of the available theoretical and empirical literature relevant to the evolution of life-histories, a number of important issues have emerged which are in need of further empirical investigation. These issues, which are not mutually exclusive, are best presented as a series of questions which can then be experimentally investigated. These questions are:

a) Can life-history tactics be detected by intraspecific population comparisons?

b) Can any observed genetic differences between geographic populations be attributed to the process of natural selection, and further, can the important ecological selection pressures be identified?

c) Is there significant additive genetic variation in individual life-history traits within natural populations and what are the consequences if significant heritabilities are found?

d) What are the genetic interrelationships between the different life-history characters or fitness components?

   i) Do "trade-offs" and specifically "reproductive costs" occur in natural populations?

   ii) Is antagonistic pleiotropy responsible for the maintenance of additive genetic variation in life-history traits?

e) Are there biologically important genotype-environment interactions, and if they do exist, what is their role in the maintenance of additive genetic variation?

The empirical literature covering the above issues is currently inadequate for a number of reasons: (1) many studies have used long-established, potentially inbred, laboratory populations rather than natural populations or populations recently derived from the wild; (2) few studies have unambiguously separated the effects of genotype and environment which is necessary for calculating quantitative genetic parameters; (3) often the traits measured simply are not components of fitness; (4) most studies have usually considered single fitness traits or only a few early life-history traits; (5) phenotypic correlations, rather than genetic correlations, are frequently used to describe the relationship between different life-history traits; (6) experiments are often performed under only one set of environmental conditions, ignoring the potential for genotype-environment interaction, or at least restricting any inferences made to the environment in which the measurements were taken; and (7) even though numerous quantitative genetic studies reported in the literature are methodologically sound, they have frequently used insufficient individual observations (small sample sizes), which have led to large standard errors.

In response to the above questions, two separate, but complementary experiments were undertaken to address specific areas concerning life-history evolution in natural populations. These two empirical studies will be referred to as the "NESTED" and "CLINAL" experiments.

The purpose of the Nested experiment was to estimate and compare, by a quantitative genetic analysis, the additive genetic variances (heritabilities) and covariances (genetic correlations) of several life-history traits measured on females from two geographically distinct natural *T. castaneum* populations. This information will allow inferences regarding the potential to respond to natural selection as well as the past selective regimes experienced by these populations. The comparison between populations from putatively different selective regimes can be used to determine if life-history tactics or variation exists at the intraspecific level. Information about the relationships between the different life-history characters, expressed as genetic correlations, is used to test for the presence of trade-offs, and more specifically reproductive costs. These genetic correlations also are used to test for the occurrence of antagonistic pleiotropy and its potential role in maintaining additive genetic variation within natural populations. In addition, the experiment was conducted under two constant laboratory temperatures to investigate the importance of genotype-environment

interaction in the evolution of life-histories. More specifically the Nested experiment evaluates the potential role of genotype-environment interaction in maintaining additive genetic variation, and the stability of the quantitative genetic parameters under different environments.

An examination of genetic differentiation between adjacent populations distributed along an environmental gradient can be particularly informative, not only when attempting to detect the influence of natural selection on the life-history of a species, but also in identifying the selective forces responsible for determining variation in fitness components. If environmental factors which vary over the range of a given species determine the relative fitnesses of genotypes, then correlations may be expected between a character gradient and a gradient in the environment (Tomaszewski et al. 1973).

This approach was adopted in the Clinal experiment which examines genetic variation in certain early life-history traits among 34 geographically separate populations of *T. castaneum* that were sampled along a latitudinal gradient in Eastern Australia. Although the environmental variables of the microhabitats experienced by the life-stages of *T. castaneum* are not known for the populations sampled, they should be at least correlated with the ambient climate.

The association between genetic variation and factors associated with geographical origin was estimated by regression techniques. For each life-history trait, population means are regressed separately (simple linear regression) and then simultaneously upon several measures of geographic position and climatological factors (multiple linear regression). The relationships between the different life-history traits are investigated by intra-locality and inter-locality correlations. Unlike the Nested experiment, the Clinal experiment assays both sexes for several life-history traits to determine the importance of genotype-sex interactions. The ecological relevance of temperature effects, expressed as genotype-environment interactions, are assessed by replicating genotypes over five constant laboratory temperatures that are representative of those that the beetles experience in nature.

Samples of the two populations used in the Nested experiment are also included in the Clinal experiment, to allow further evaluation of the adaptive significance of life-history differences between the two populations. The consistency in performance of these populations with observed latitudinal or climatic trends is examined. The ability of *T. castaneum* to fly and the seasonal movements of grain, a common habitat, could mean these two populations were not representative of their respective localities.

2.2 The Model Organism

The 'rust-red flour beetle' *Tribolium castaneum* (Herbst) is a well known pest of stored grain and cereal products, and has probably lived in human grain and food stores since early historical times (see Sokoloff 1974 for review). Taxonomically, the genus *Tribolium* belongs to the family *Tenebrionidae* of the order *Coleoptera* (Hinton 1948). The geographic distribution of *T. castaneum*, like the ecologically similar 'confused flour beetle' *T. confusum* (Duval), is cosmopolitan since they are easily distributed from one place to another in stored food products (Champ and Dyte 1976; Good 1936; Hinton 1948).

While both species have been extensively used in genetic and ecological studies, *T. castaneum* has been used more frequently in quantitative genetic studies (Bell 1982). *Tribolium castaneum* was chosen in preference to *T. confusum* as the experimental organism for this research, not only for its suitability as a quantitative genetic model, but also because of its greater importance as a pest species in Australian grain storages (Champ and Dyte 1976; Greening 1980; Johnston 1981; Miller 1984).

In Australia, the grain handling environment produces desirable temperatures for the growth of grain insect populations mainly because grain is harvested at near optimal temperatures for growth of grain insect populations (approximately 34°C for *T. castaneum*); the grain in storage remains at high temperatures for long periods; and throughout the year in grain growing areas, localized temperatures in potential grain insect habitats remain well within the range needed for insect survival and reproduction. Therefore, within Australia, the number of suitable winter refuges, the number of insects surviving in them and the rate of reproduction of these populations is largely governed by food availability (Johnston 1981).

With appropriate experimental design, the genotype, environment and genotype-environment interaction variance components can be estimated for a given population (Falconer 1981). To separate these components, it is necessary to satisfy two requirements common to all genetic analyses of quantitative traits:

a) groups of relatives must be recognizable; and

b) the effects of genotype and environment on the phenotype must be separable, in a statistical sense (Lawrence 1984).

However, when attempting to measure the quantitative genetic architecture of populations remaining in their natural habitat, it is difficult, if not impossible for most species, to

recognize the genetic relationships between individuals (Giles and Edwards 1983). Further, in the natural habitat of a given population, differences between individuals due to genetical causes are usually completely confounded with differences that are due to the environment, making it impossible to separate the genotypic and environmental components of phenotypic variation. Furthermore, it is usually impossible or impractical to measure life-history traits on individual organisms, including flour beetles, without interfering with their normal activities. Therefore, it is generally necessary to conduct quantitative genetic experiments under uniform environments such as in the laboratory.

The idea that populations freshly sampled or recently derived from the natural habitat and assayed under laboratory conditions more accurately reflects the state of natural populations is appealing (for example Giesel 1986b; Giesel et al. 1982a,b; Mitchell-Olds 1986; Murphy et al. 1983; Palmer and Dingle 1986; Travis et al. 1987). However, the extent to which inferences about variation and its selection in the natural habitat, can be made from the observation and analysis of material raised in the laboratory, is open to debate (Barker and Thomas 1987; Clark 1987; Giles and Edwards 1983; Istock 1982, 1983; Lawrence 1984). The performance for quantitative characters of material reared in the laboratory environment may be quite different from the expression of the same genotype in the natural habitat. Should the rank order of genotypes change between the natural habitat and the laboratory, then extrapolation may not be valid. One method of investigating this problem is to measure the performance of different genotypes in several different laboratory environments. If genotype-environment interaction is present, and due to a change in genotype rank, then caution should be exercised when drawing inferences (Lawrence 1984).

Ideally, the laboratory environment of the species in question should closely reflect the conditions of its natural habitat. It can be argued that the standard medium used for laboratory rearing of *T. castaneum* constitutes a near natural environment, because of the beetles' association with stored grain products (King and Dawson 1972; Mertz 1972). This similarity between the laboratory environment and the natural habitat means that major adaptive challenges can be avoided when natural populations are introduced to the laboratory. This advantage and the wide geographic distribution of *T. castaneum* satisfy the major requirements, suggested by Hegmann and Dingle (1982), of a good model organism for establishing the genetic basis of variation in life-history traits in natural populations.

Under natural conditions, *T. castaneum* displays many of the life-history attributes of a colonizer: high intrinsic growth rate, rapid development and early maturity, high ratio of

the rate of increase to the risk of mortality, excellent dispersal ability, and strong predatory tendencies (Dawson 1977; Mertz 1971). Therefore, at least in this species, the criticisms of Service and Rose (1985), that novel environmental effects can produce artifactually positive correlations between life-history traits in populations subjected to the laboratory environment for only a few generations, should be inapplicable, because:

1) the laboratory and natural environments are similar; and

2) the species frequently experiences colonizing episodes in nature, so that the life-history of *Tribolium* should be adapted to encountering novel environments.

Not only is *T. castaneum* a good model organism for the above reasons, but also because of the many other technical and biological benefits associated with its use. Such advantages include:

1) Natural populations are easily sampled from their wild habitats. Potential habitats can be readily located, because they are usually man-made.

2) Laboratory stocks need little care and large outbred populations are easy to maintain (Haskins 1975; Sokoloff 1974).

3) The rearing medium is uncomplicated and easy to prepare. Diets of grain flour supplemented with dried brewers' yeast or vitamins are suitable (King and Dawson 1972; Sokoloff 1974).

4) They have high rates of reproduction — individual females laying up to 15 eggs per day are not uncommon (Bhat and Bhat 1974b). This makes the replication of genotypes or groups of relatives needed for quantitative genetic experiments relatively simple.

5) *Tribolium* have long life-spans, 6-9 months under favourable conditions (Haskins 1975). As compared with *Drosophila*, the relatively long generation interval and long adult life-span have usually been considered disadvantageous because they lead to experiments of long duration. However, these qualities mean that census taking and attendant handling can be at more infrequent intervals than with *Drosophila*, and occupy a smaller fraction of the total lifespan. Further, the infrequent censuses enable greater experimental replication (Mertz 1972). Also, the extreme longevity of adults is advantageous for age-distribution studies (Mertz 1969).

6) Although the generation interval is long as compared with *Drosophila*, monthly generation cycles are easily obtainable under optimal conditions of temperature, humidity, nutrition and density (Bell 1982), thus making *T. castaneum* a suitable organism for quantitative genetic studies.

7) The sexes are easily identifiable in both the pupal and adult stages, facilitating collection of virgin females.

8) Live adult insects are easily handled in the laboratory as they are unable to climb smooth vertical glass surfaces or fly at temperatures below 25°C (Haskins 1975).

9) The genome is suitable for quantitative genetic studies. In *T. castaneum*, there are ten pairs of chromosomes, including XY sex chromosomes, and genetic recombination in both sexes. This offers advantages over *D. melanogaster* which have only 3 pairs of large chromosomes (Bell 1982: King and Dawson 1972).

10) Unlike *Drosophila*, *Tribolium* displays somatic cell division in the imaginal stages (Bozcuk 1972: Day and Powning 1949). Therefore *T. castaneum* is probably a more useful experimental organism for investigating adult life-history traits, since most higher organisms also display somatic cell division in the adult.

11) All life-history stages (eggs, larvae, pupae and adults) can readily be recovered and characterized by passing the dry, presifted culture medium through sieves of appropriate pore size. This sieving of the life-history stages seems to produce only small adverse effects if the censuses are spaced widely enough in time (Mertz 1972).

12) Polygamous mating habits make it an ideal organism for nested designs.

13) The physical environment (conditions of light, temperature and humidity) can be easily controlled by using suitable laboratory incubators.

14) The small size of flour beetles allows husbandry containers to be very compact and since the media assumes the shape of the container, the geometry of the environment is easily controlled and replicated. Further, many experimental units can be randomized and stored within a single incubator.

15) There are numerous genetic markers, including a black mutant (Sokoloff 1966, 1977).

16) Maintenance costs are reasonably low.

Obviously not all attributes of *T. castaneum* are favourable. One disadvantage is the fact that adults and larvae display cannibalistic tendencies, eating the less mobile eggs, pupae and callows (very young adults with soft exoskeletons) (see Craig 1986; Englert and Raibley 1977; Lang and Mertz 1982; Mertz and Robertson 1970; Sokoloff 1974). Another problem is that the beetles are subject to parasitism (especially sporozoans) and disease (King and Dawson 1972; Sokoloff 1974, 1975). Unattended cultures have a tendency toward "conditioning" of the medium by the accumulation of wastes and/or quinones secreted by the odoriferous glands, which may affect productivity of adult beetles or may produce teratological specimens if the quinones come in contact with the immature stages at critical periods of development (Sokoloff 1974, 1975). Such conditioned media can also lead to the experimentalist developing an allergy (King and Dawson 1972). Although pre-adult size and developmental stage are fair indications of age, the age of adult *Tribolium* cannot be diagnosed (Mertz 1972). Also, the burrowing habits of flour beetles makes behavioural observations especially difficult. Many of these problems can be overcome or minimized by appropriate laboratory husbandry procedures.

2.3 General Materials and Methods

Location of the Experiments and the Laboratory Environment. Following the introduction of natural populations, all experimental work was carried out in the Genetics Research Laboratories, located at the University of New England, Armidale.

The beetles were cultured under controlled environmental conditions — with temperature and humidity being the most important for *T. castaneum* (Sokoloff 1974). The experimental cultures were kept in darkened, fan-forced laboratory incubators, preset and monitored at the desired temperature (see separate experiments for details). For the Clinal experiment this included a refrigerated incubator. The performance of the incubators was monitored daily. Minimal aeration, by a small opening at the top of each facilitated convection and removal of CO_2.

In incubators, the relative humidity is conveniently controlled by the use of saturated salt solutions (Carr and Harris 1949; O'Brien 1948; Solomon 1952; Stokes and Robinson 1949). A saturated solution of sodium nitrate ($NaNO_3$) was placed in the bottom of each incubator to standardize the relative humidity. Although sodium nitrate maintains a relative humidity

slightly above 70%, the condition that is standard (after Sokoloff 1977) and near optimal for egg lay (Orozco and Bell 1974), no adverse effects on the rate of development, oviposition or mortality were expected (Howe 1956, 1962). Also the expected relative humidities will vary slightly (3–4%) over the range of temperatures used (21°–37°C). However, these differences were considered to be unimportant relative to the effects of temperature on the life-history of *T. castaneum*.

The time spent out of the incubators during laboratory handling (at 21°C and 60–80% r.h.) was standardized, and at all times kept to a minimum.

Experimental Containers for Culturing the Beetles. Single beetles or pairs of beetles were kept in glass vials (7 ml) with perforated plastic caps and containing up to 2 g of medium. For larger groupings, either vials (30 ml), 1/4 pint creamers or juice bottles (250 ml) containing up to 10 g, 50 g and 100 g of medium respectively, and closed with plastic foam stoppers, were used. Stocks and mass cultures were kept in plastic containers (13.5 x 9.5 x 7 cm) with perforated lids, and containing up to 400 g of medium.

The Culture Medium. The standard culture medium consisted of whole-wheat flour fortified with 5% (by weight) of dried, powdered brewers' yeast.

To maintain constancy of experimental conditions, sufficient flour for all the experimental program was obtained from the one source before the study commenced. Ben Furney Flour Mills of Dubbo, N.S.W., in February 1984, supplied 550 kg of flour, specifically untreated, and milled from a blend of 40% NH_2 grade (82–83 pool, Trangie origin, 10.6% protein, varieties — Shortim 35.4%, Banks 34.9% and Cook 29.7%) and 60% ASW grade wheat (83–84 pool, Ballimore origin, 12.7% protein, varieties — Banks 80.0% and Kite 20.0%). The flour was sifted through a 210 μm aperture mesh sieve with the aid of an Endecott's EVSI variable speed, intermittent motion test sieve shaker. This was to remove any foreign material or organisms (approximately 2 individuals of *T. confusum* or *Ephestia* spp. per 100 kg were detected), and to reduce the particle size sufficient for the collection of eggs. The flour was then sterilized at 60°C for eight hours (Sokoloff 1977), with two-hourly stirrings, before being sealed in 7–8 kg lots in sterilized aluminium cans and stored at low temperature.

The dried brewers' yeast powder, supplied by Healtheries Ltd. of Auckland N.Z. was derived from *Saccharomyces cerevisiae*. Again sufficient quantities for all the experimental

work was purchased, blended and stored in the dark at low temperature.

The flour was thoroughly mixed prior to use with the required weight of yeast (19 parts flour : 1 part yeast) in 2 litre sterile glass jars. The quantities needed were measured into the desired culture containers with the aid of a Mettler PC 4000 balance, or for small quantities (2 g or less), using predetermined volumes. Any variation in the allotted quantity of medium to individual culture containers was small and expected to be random. All culture media were conditioned at the appropriate experimental temperature and relative humidity for at least 24 hours prior to use.

Separation and Handling of the Life-Stages. Samples of eggs were collected by allowing fertile females to lay eggs in a quantity of fresh medium for a fixed time interval. The females (and males) were then removed, and the culture medium containing the eggs sifted through a mesh sieve with an opening of 210 μm. This aperture size allows the presifted medium to pass through the sieve, but retains the larger eggs. For counting, these eggs were transferred with the aid of a camel hair brush to a linear egg counting stage (after Muir and Grossman 1973), where they could be spread out by gentle tapping, then placed under a binocular dissecting microscope (Olympus model SZ-III) at low magnification.

The other life-stages were similarly retrieved from the culture medium by using sieves with the appropriate aperture size (see Sokoloff 1974). Adults, pupae and large larvae are retained by a mesh sieve with an opening of 500 μm. A watch-glass, camel hair brush and glass petri dishes were used for handling and sorting these life-stages. When necessary, a Mettler HL52 microbalance (reproducibility $\pm$ 0.02 mg) was used for weighing individual beetles.

Sexing of Beetles. Males and females are readily distinguished in either the pupal or adult stages. When the ventral posterior of the male and female pupae are examined under a binocular dissecting microscope at low magnification, the female has a pair of prominent genital lobes on the terminal segment anterior to the cerci, which are reduced to indistinct elevations in the male (see Anon. 1960).

There are two efficient procedures available which are effective in sexing *T. castaneum* adults. The first method, which involves the application of gentle pressure to the abdomen to expose the genitalia (King and Dawson 1972) was not used due to the risk of physical injury to the beetles. The second, and more desirable method, involves checking the ventral

surface of the prothoracic legs for the secondary "sex spot" (a sub-basal setiferous puncture) which is present in males and absent in females (Faustini 1982; Hinton 1942). To immobilize the adults, rather than using ether, they were placed on a smooth surface made of plaster of Paris set in a small petri dish, which was itself cooled by a bed of ice contained in a larger petri dish. The cold immobilizes the adults temporarily with no ill effects. An optical fibre was used to avoid any heat from a light source while under magnification.

Species Identification. After collection, and before being brought to the laboratory, all putatively wild caught adult flour beetles were immobilized on a cold surface, individually checked and classified according to species. Adults of *T. castaneum* and *T. confusum* are readily distinguished under a dissecting microscope 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 1948; King and Dawson 1972). Species other than *T. castaneum* were discarded.

The Use of Natural Populations. Both the Nested and Clinal experiments made use of "natural" populations, where F_3 descendents of wild caught adults were assayed. Before attempting to separate the effects of genotype and environment on the phenotype, 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). During these two generations each population was maintained as a large outbreeding culture. However, it must be remembered that all genetic studies of natural populations are subject to a possible bias due to drift or selection when the offspring of wild individuals are kept for several generations in the laboratory (Capy et al. 1983).

Control of Disease and Parasites. There are a number of disease organisms and parasites (including *Farinocystis tribolii*, *Adelina tribolii*, psocids, and mites) that are capable of seriously affecting *T. castaneum* cultures (Sokoloff 1974).

To prevent the transmission of these organisms into the laboratory from wild caught populations, samples of eggs (F_1 generation) from each population were collected *en masse* and washed in a solution containing 0.1% benzalkonium chloride (supplied by Sigma Chemical Company Inc.) in distilled water, following the method of Winks (1981). Any stocks maintained in the laboratory at the time were also subjected to this procedure. The washing

procedure was carried out externally to the Genetics Research Laboratory, and all incubators and equipment were rigorously sterilized before introducing the washed eggs.

General hygiene in the laboratory included the heat sterilization of all metal and glassware (200°C for 1 hour). Benches on which cultures were handled were kept clean and swabbed with ethanol after use. Equipment that could not be heat sterilized (including plastic caps and containers) were thoroughly washed under hot water and liberally rinsed with ethanol. Plastic foam stoppers were used on one culture only before being discarded. Although some wild caught samples were infested with mites prior to egg washing, no evidence of diseases or parasites was detected over the course of the experiments.

The Black Mutant. Bhat and Bhat (1974a) demonstrated that the injection of semen, and not just the physical presence of males was necessary for enhancing egg production in *T. castaneum* females. In both the Nested and Clinal experiments, males from an unrelated black mutant stock were used to mate with females assayed for fecundity. This was done to standardize male effects over the different genotypes and populations. The original stock was supplied by Dr. DuWayne C. Englert, Dept. Zoology, Southern Illinois University, Carbondale, Illinois 62901 U.S.A., in July 1982 and subsequently maintained in large numbers in 400 g medium, changed monthly.

The use of this black mutant made the identification of the sexes during fecundity assays very simple, and dead males were easily identified and replaced. Use of the black body colour mutant was more suitable than identification by antennal clipping (Ruano, Barrera and Orozco 1970).

Computing Facilities and Software. All computing was done on the DEC20 computer at the University of New England using various packages and programs. These included programs from the BMDP (Dixon 1983) and SPSS^x (SPSS Inc. 1983) statistical packages, and the LSML76 (Harvey 1982) and REG (Gilmour 1985) programs. Specific details will be given in discussing the separate experiments.