The Cost of Reproduction in *Callosobruchus maculatus*

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And was Jerusalem builded here among these dark cephalic shields?

(William Blake, 1757-1827)

Acknowledgements

When I was warned that this would be the most difficult bit, I didn't realise that it would be because every name that hurtled out of the shadows demanding inclusion would drag with it a jumble of ill-assorted memories. How should I work - seeing Imogen 12 h after emergence, Ben watching mist-nets from the door of the caravan in Whirlow Park, Guy confidently finding a wall end-on in the swirling clouds of Cader Idris, Paul's "I've gone to lunch with the delightful Emily" note, Norma's attempts to dissolve B2:209's handle, meeting Gail in the Age Concern café or exchanging shells after a sloe gin or two - into flowing prose. I have tried to pick out the people who have helped me most during the last three years. I suspect that I will have left a few of you out; if I have, I'm sorry.

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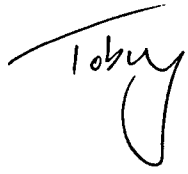

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Summary

The bruchid beetle *Callosobruchus maculatus* does not feed as an adult. The resources such as energy or nutrients available to it are therefore fixed at emergence. Allocation of resources to processes contributing to one trait must therefore reduce those available for allocation to others and trade-offs between these traits are expected. The present study investigates the trade-off between current and future reproduction, the cost of reproduction, in both females and males of this species taking an explicitly phenotypic approach.

The trade-off between adult longevity and lifetime fecundity in females is demonstrated using experimental manipulations of fecundity. It is shown that reduced adult longevity reflects reduced future reproduction and that this trade-off is therefore the cost of reproduction. The inadequacy of phenotypic correlations in demonstrating this trade-off is shown.

The allocations of dry weight, water, lipid and energy by females to reproductive and non-reproductive processes are measured. For each resource, the predicted slope of the trade-off between adult longevity and lifetime fecundity is calculated based on that resource being limiting. From a comparison between these predicted slopes and the slope measured using experimental manipulation, it is concluded that none of these resources can be rejected as the limiting resource and that they all may contribute to the trade-off.

Males make a non-trivial investment in ejaculate. This investment reduces a male's future reproductive success. To some extent these costs are short-term; the male soon regains his fertility. However, there are also long-term costs due to reduced longevity.

The fitness consequences of remating to females are investigated. Fecundity is increased following remating but it is impossible to distinguish between the nutritional and manipulative roles of the ejaculate.

Finally, the costs of reproduction are summarised and their generality and implications discussed.

Contents

1. Introduction

1.1. Life history theory

1.1.1. Life-history theory

Natural Selection acts to maximise the rate of increase of alleles. The only two properties of organisms which are directly related to the rate of increase of alleles are age-specific values of fecundity and mortality (Lessells, 1991); other traits act indirectly through their effects on these two traits. Schluter *et al.* (1991) formally defined a life-history trait as any character correlated with total fitness when all other traits are held constant. Life-history theory is concerned with how constraints lead to optimal combinations of these life-history traits (Smith, 1991). The relationship between these traits and the the intrinsic rate of increase of a genotype, r, is expressed in the Euler-Lotka equation (Lotka, 1907).

$$1 = \sum_{x=1}^{\infty} l_x m_x e^{-rx} \qquad (1)$$

Where l_x is survival to age x and m_x is the number of female offspring produced at that age.

In practice, the intrinsic rate of increase of a genotype is difficult to measure because the genotype is not expressed by an organism. The phenotype is determined by the poorly understood interactions between an organism's environment and its genotype. A more tractable and equivalent equation relates the two traits to the expected number of descendents that a female of a given age is expected to have in future generations, her reproductive value, RV (Fisher, 1930):

$$RV_x = \sum_{y=x}^{\infty} (l_y/l_x) \cdot m_y \cdot e^{-r(y-x+1)} \qquad (2)$$

where l_y/l_x is the probability of a female surviving from age x to age y, m_y is her fecundity at that age and $e^{-r(y-x+1)}$ weights offspring by how far in the future they are produced. This equation is easily broken down into terms representing present and future reproduction (Williams, 1966):

$$RV_x = m_x \cdot e^{-r} + \sum_{y=x+1}^{\infty} (l_y/l_x) \cdot m_y \cdot e^{-r(y-x+1)} \quad (3)$$

1.1.2. Trade-offs

The above equations define fitness in terms of a number of fitness components, or life-history traits. One might predict that natural selection would maximise fitness by maximising each life-history trait. However, natural selection has not led to the evolution of life histories in which all life history traits are maximised (Partridge and Harvey, 1988). The only plausible explanation is that there are trade-offs between life history traits (Rose, 1983). Trade-offs are the negative relationships between life history traits, such as clutch size or longevity, which occur because of resource limitation. Animals have a finite amount of resources, such as energy or time, available to them. Consequently, if resources are allocated to one life history trait there are fewer available for allocation to others.

There is considerable confusion over the definition of trade-offs. For example, Stearns (1992) confusingly defines three sorts of trade-offs: physiological trade-offs, 'caused by allocation decisions between two or more processes that compete directly with one another for limited resources within a single individual'; microevolutionary trade-offs, 'defined by the response of populations to selection' '...when a change in one trait that increases fitness is linked to another trait that decreases fitness' and macroevolutionary trade-offs, 'defined by comparative analysis of variation in traits among independent phylogenetic events'. What Stearns refers to as physiological trade-offs are trade-offs as they are usually defined (*e.g.* Rose, 1983; Pease and Bull, 1988), although some authors have drawn a distinction between trade-offs with ecological and physiological causes (Calow, 1979; see below). What Stearns refers to as microevolutionary trade-offs are not trade-offs in the usual sense, but genetic correlations which, given certain increasingly undermined assumptions (see below), may be used to measure trade-offs. Finally, what Stearns refers to as macroevolutionary trade-offs are phenotypic correlations measured between taxa. Given the considerable logical difficulties in demonstrating trade-offs from phenotypic correlations measured within a population of a

single species, it is doubtful whether such data could even be used to demonstrate trade-offs (Partridge and Harvey, 1988; Lessells, 1991).

1.1.3. The cost of reproduction

If fitness cannot be increased indefinitely, any increase in an animal's current reproduction must be accompanied by a reduction in its potential for future reproduction (from equation 3). This negative relationship between current and future reproduction is the cost of reproduction (Williams, 1966). It is useful to draw a distinction between costs of reproduction which have a physiological basis and those which have an ecological basis (Calow, 1979). Physiological costs of reproduction occur because animals have a finite amount of resources, such as time, energy or nutrients, available to them; if they allocate resources to increasing current reproduction they will have fewer resources for allocation to later reproduction. Ecological costs occur if increasing current reproduction increases an animal's exposure to environmental risks, such as the risk of predation. These concepts will be discussed in greater depth in Chapter 4.

The cost of reproduction may be invoked to explain some of the more intractable problems in biology. For example, it may explain why animals often restrict their current reproductive output to below that which maximises their fitness from that breeding attempt (Charnov and Krebs, 1974). Also, the shape of the trade-off between current reproduction and future reproductive potential determines the optimal amount of current reproductive investment. Age-related changes in this shape may explain observed age-related variation in reproductive investment, and in particular whether animals should be iteroparous, reproducing repeatedly, or semelparous, reproducing once (Pianka and Parker, 1975; Pianka, 1976). The arguments of Pianka may be extended to explain variation in patterns of reproductive effort between environments, between taxa or between individuals within a species.

The cost of reproduction may also explain the evolution of senescence. The values of adult life-history traits may decline later in life either due to the accumulation of damage suffered earlier in life or due to senescence, innate deterioration of the body. Senescence is

maladaptive; it could evolve because natural selection is slower to remove deleterious mutations acting later in life than those acting earlier. This differential rate of elimination of age-specific mutations would lead to an accumulation of mutations having a deleterious effect later in life (Medawar, 1952). Alternatively, due to the cost of reproduction, genes which increased resource allocation to early reproduction would reduce the resources available for other functions. This reduction in resource availability might result in a lack of somatic maintenance and consequently an increase in the rate of senescence (Kirkwood, 1981; Partridge, 1987). In support of this hypothesis, Kirkwood and Rose (1991) have derived models which suggest that, in order to maximise fitness, the optimal investment of resources to somatic maintenance may be less than that required to prevent senescence.

1.1.4. Measuring trade-offs

One of the major controversies over life history trade-offs is how to measure them (Reznick, 1985; Bell and Koufopanou, 1986; Pease and Bull, 1988; Stearns, 1989; Partridge, 1992). While the existence of trade-offs seems inevitable (Partridge and Harvey, 1988), the likelihood of detecting them depends upon the methods used (Bell and Koufopanou, 1986; Lessells, 1991). There are two approaches to measuring trade-offs, the phenotypic and the genetic approaches, outlined below.

i. The phenotypic approach

The most obvious method of demonstrating trade-offs seems, at first sight, to be to measure the observed relationship between traits in a population. If there were a trade-off between two traits one might expect this relationship, known as a phenotypic correlation, to be negative. However, the phenotypic correlation depends upon more than the trade-off because individuals differ not only in the allocation of the resources they have, but also in the total amount of resource.

Individuals with abundant resources could allocate more to all life history traits than impoverished individuals, so that, if individuals differ greatly in the resources available to

them, the phenotypic correlation between life history traits may be positive. The relationship observed will depend on whether there is more variation in allocation or in resources available. Any trade-off may be masked because of a large variance in resource availability (van Noordwijk and de Jong, 1986; Stearns, 1989). There are two phenotypic methods of avoiding this problem.

The first is to control statistically for the confounding effects of resource availability variation (*e.g.* Clutton-Brock *et al.*, 1983). To do this, the availability of the limiting resource to individuals and its relationships with the two traits must be measured. Then a statistical technique such as partial correlation is used to calculate the relationship which remains between the two traits when the confounding effects of resource availability variation are removed. However, this approach is dependent on both knowledge and accurate measurement of the limiting resource or some indicator of this resource such as body weight. The argument is circular; the trade-off may only be measured with knowledge of the limiting resource yet the limiting resource may only be determined from measurement of the trade-off.

In the second method, experimental manipulation, individuals from a population are randomly allocated to different groups, so that each group has the same mean level of resources. The groups are manipulated so that they differ in how individuals allocate these resources to a particular life history trait. Thus, between-group variation in resource availability is eliminated while between-group variation in the way that these resources are allocated to life history traits is increased. Because variation in resource availability has been eliminated, the relationship between group means must be due to variation in resource allocation; a negative relationship shows that there is a trade-off. This interpretation depends on the assumption that the manipulation directly affects only one of the traits and that the response of the second trait is due entirely to an indirect response because of the trade-off (Reznick, 1985; Bell and Koufopanou, 1986).

Reznick (1985, 1992a,b) refuses to accept that experimental manipulations are a valid method of measuring the cost of reproduction because they may involve different mechanisms from those revealed by genetic studies:

> "The genetic mechanism underlying the response in Rose and Charlesworth's [1981a] selection experiment is therefore different from the phenotypic plasticity in lifespan revealed by Partridge and her colleagues' manipulations of mating frequency [Partridge and Farquhar, 1981; Partridge *et al.*,1986; Partridge *et al.*, 1987; Fowler and Partridge, 1989]. It thus appears that Rose and Charlesworth's original result was indeed an example of antagonistic pleiotropy, rather than selection for a change in mating behaviour." (Reznick, 1992a).

The implication is that phenotypic plasticity has no relevance to, and moreover obscures, the antagonistic pleiotropy that is the 'real' cost of reproduction. Quite apart from the relevance or otherwise of mating behaviour (Partridge, 1992; Reznick, 1992b), phenotypic plasticity is relevant for two reasons. First, a gene for the presence or absence of phenotypic plasticity might be fixed. However, there are physiological pathways which control both the perception of the environment and, in response to the action of the genes controlling phenotypic plasticity, the response to that environment. These physiological pathways are genetically determined and hence subject to evolution by natural selection (Houston and McNamara, 1992). If genetic correlations did not measure phenotypic plasticity, this would limit their usefulness in predicting evolutionary trajectories. Second, if animals are already capable of responding to a novel environment in an appropriate way due to phenotypic plasticity, it seems unlikely that there would be any developmental or genetic constraints to prevent the evolution of this response following selection.

A further, more serious criticism of experimental manipulations is that they might alter the options set available to an individual. The options set is the set of evolutionary options open to an individual; its boundary is the trade-off curve (Sibly and Calow, 1986). If the options set were altered, the measured trade-off curve would not reflect the trade-off curve of the population from which the manipulated individuals were taken. To minimise this problem, manipulations should only reflect variation which is likely to occur in the natural environment.

ii. The genetic approach

Attempts have also been made to measure trade-offs using a genetic approach (Rose and Charlesworth, 1981a,b; Møller *et al.*, 1989a; Partridge and Fowler, 1992). In a constant environment, a negative genetic correlation is expected between the traits if there has been a trade-off between them (Falconer, 1989). Selection will fix pleiotropic genes which have a positive effect on both traits and eliminate those which have a negative effect on both traits. Alleles which have a positive effect on one trait and a negative effect on the other suffer less intense directional selection. They tend to persist at intermediate frequencies and are expected to constitute the bulk of the genetic covariation between the two traits. Consequently, the existence of a negative genetic correlation between two traits is evidence for the existence of a trade-off.

Genetic correlations are needed if the aim is to predict the response to selection (Reznick, 1985) and may be the only possible approach where direct manipulation of traits is impossible, for instance in studies of the trade-off between egg size and clutch size (*e.g.* Ovenden, 1991). However, there are many unresolved problems with the interpretation of genetic correlations and they do not consistently reveal trade-offs. One reason for this may be that selection does not always lead to the fixation or elimination of alleles having a positive or detrimental effect on both traits. This might be due to alternating selection pressures due to environmental fluctuation (Stearns, 1989), no net selective pressure due to the cancelling out of opposing genetic correlations (Pease and Bull, 1988; Charlesworth, 1990) or sufficiently high mutation rates to counter the selection pressure (Charlesworth, 1987; Houle, 1991).

Two potential problems with genetic correlations as a method of demonstrating trade-offs are that tediously large sample sizes are needed for their estimation and only a narrow range of variation is examined whereas experimental manipulations permit a wider range of allocation strategies to be examined (Partridge and Sibly, 1991; but see Chapter 3).

1.2. *Callosobruchus maculatus*

1.2.1. Life cycle

Females of the bruchid beetle, *Callosobruchus maculatus* (F.), have a lifetime fecundity of up to 120 eggs. Eggs are laid on the testa of host beans (for a description of egg laying decisions see Wilson, 1988). Each egg is attached to the testa by a rapidly-drying, adhesive-containing fluid coating, spumaline (Hinton, 1981; Credland, 1992). After approximately four days the dark cephalic shield of the developing first instar larvae is clearly visible beneath the transparent egg membranes (for a description of larval development see van der Meer, 1979). By the seventh day the larva has burrowed through the egg membranes and testa into the cotyledon. Usually the egg membrane changes colour as it is filled with white or brown displaced material from the bean. However, larvae occasionally burrow into a bean without filling the egg membranes with displaced material. Larvae are unable to move from one seed to another; larvae cohabiting within a bean do not attack each other but exhibit scramble process competition (Smith and Lessells, 1985). After three or four instars (Bellows, 1982a) the larva pupates immediately below the testa. The pre-emergent adult is visible beneath a translucent area of the testa. Three to four weeks after oviposition the adult beetle leaves the pupa, cuts a small circular hole through the testa and emerges from the bean. Adults may mate within seconds of emergence from the bean. They may mate several times over a period of days (see Chapter 6). Females have been shown to produce attractant pheromones (Qi and Burkholder, 1982) and there is a well defined courtship ritual (Rup, 1986).

The effects of environmental conditions on life history parameters are well described (Temperature and humidity: Schoof, 1941; El-Sawaf, 1956; Howe and Currie, 1964; Giga and Smith, 1983; 1987. Host seed availability: Credland, 1986. Larval density: Giga and Smith, 1981; Smith and Lessells, 1986; Messina, 1991. Host seed variation: Nwanze and Horber, 1976; Giga and Smith, 1981. Adult density: Bellows, 1982. Adult nutrition: Larson and Fisher, 1938. Mating frequency: Brauer, 1944; Ouedraogo, 1978). Details of the culturing conditions at Sheffield are described in the next chapter.

1.2.2. The general biology of *Callosobruchus maculatus*

C. maculatus is well suited to a study of trade-offs. There are many practical advantages of working with this species: it is easy both to maintain populations in the laboratory and large numbers of virgin adults are easily obtained (see Chapter 2); males and females are unambiguously and quickly distinguished by their elytral markings (Southgate, Howe and Brett, 1957); virgin adults rarely refuse to mate for longer than a minute or two. Successful copulations can be distinguished from unsuccessful mounting attempts. Both female fecundity and male fertility are easily measured.

As well as being easy to work with, many aspects of its biology make this species particularly suitable for studying trade-offs. There is wide variation in most aspects of the life cycle; this variation can be influenced by controlling a variety of environmental parameters (see above). Individuals obtain the resources that they need to complete their life cycle while they are larvae and are given no opportunities to feed as adults in the laboratory. While adults are able to feed on sugar solutions, and have been reported to feed on nectar in the field (Larson and Fisher, 1938), they would have no opportunities to feed as adults in grain stores. Consequently, adults are unable to compensate for experimentally imposed patterns of resource allocation by adjusting their food intake. Any increase in allocation to one trait must result in decreased allocation elsewhere.

1.3. Objectives

The main aims of this study were:

1. To demonstrate the cost of reproduction in female *C. maculatus* using a phenotypic approach and to compare the validity of phenotypic correlations with experimental manipulations as a means of demonstrating trade-offs.

2. To measure female allocation of resources between non-reproductive and reproductive processes and to use this information to investigate the limiting resource underlying the trade-off.

3. To measure the costs of reproduction in male *C. maculatus* using a phenotypic approach and to distinguish between the different male-female interactions causing them.

4. To investigate the possibility that females may benefit from remating in spite of the costs involved.

2. General methods

2.1. The stock culture

2.1.1. Origin of the stock culture

All experiments were carried out on a single strain of *Callosobruchus maculatus*, originally collected in 1974 from the field in Brazil by B.J. Southgate. It has been maintained at the Natural Resources Institute, Slough, UK (1974 - 1977), at Imperial College, Silwood Park, Ascot, UK (1977 - 1984) and at the Department of Animal and Plant Sciences, Sheffield since July 1984 (Wilson, 1989; Ovenden, 1991). It is the same strain as that used by Bellows (1982a,b), Wilson (Wilson, 1988, 1989; Wilson and Hill, 1989), Ovenden (1991) and Eady (1991, 1992).

A second population, derived from the Slough strain, was maintained in Reading. Black and tan morphs of *C. maculatus* were originally isolated in this Reading population by R.H. Smith (K. Wilson, pers. comm.). From these populations, pure breeding homozygous strains were isolated between September and November 1988 at Sheffield by K. Wilson (Eady, 1992).

2.1.2. Culturing methods

i. Culturing conditions

At both Sheffield and Imperial College (Bellows, 1982a) populations were kept in a constant temperature and humidity room at 30 ± 2 ^{o}C on a 16 h light: 8 h dark cycle. Humidity was maintained at 70 ± 5% r.h. (35 ± 5% r.h. for black and tan morphs). For some months during 1984 - 1986 and 1988, humidity fell to 40 - 50% r.h..

ii. Stock maintenance

The beetles were cultured on black-eyed beans, *Vigna unguiculata* (L.) Walp. obtained through a health food supplier from California, USA. They were kept in clear polystyrene boxes (273 x

152 x 102 mm). Four sub-populations were cultured one week apart. Because the beetles have a generation time of approximately 4 weeks, and because there is considerable variation in their larval developmental period, recently emerged beetles were available continuously. At one week intervals, adult beetles which had emerged during the previous week in the culture box set up 4 weeks earlier were anaesthetised with carbon dioxide. 100 - 150 of these beetles were transferred to a box containing approximately 2000 fresh beans. They were left to oviposit for one week and were then removed and discarded. This culturing procedure kept the larval densities within the beans low and hence minimised the appearence of the dispersive flight morph (Caswell, 1960; Utida, 1972; Messina and Renwick, 1985) which differs from the normal flightless morph in a range of life history characters (Utida, 1954; Ouedraogo and Huignard, 1981).

Prior to March 1988 the four sub-populations were cultured in genetic isolation from each other so that genetic divergence due to random drift may have occurred (Wilson, 1989). Since then, until March 1991, to establish a degree of gene flow between the sub-populations, 10 - 20 individuals from the culture box set up 3 weeks earlier were added to the new box, 3 d after it was set up (Ovenden, 1991).

Inevitably there will have been differences in the culturing conditions between the four sub-populations; for example, no attempt was made to regulate the time of day at which culturing took place. To minimise variation within experiments beetles which were compared came from the same cohort of the same sub-population. Occasionally, this was impossible; the reasons for, and consequences of these between sub-population comparisons are discussed in the pertinent methods sections.

2.2. General procedures

2.2.1. Obtaining virgins

To obtain newly emerged unmated adults, beans containing pre-emergent adults were placed singly in the compartments (20 x 20 x 18 mm) of clear polystyrene cell trays. The cell trays were inspected at regular intervals. Those adults which emerged alone or with others of the

same sex within a compartment were virgins and were removed and kept; other adults were removed and discarded. Unless stated otherwise, virgin beetles of the same sex were stored in groups of five for the period between their removal from the cell trays and the start of the experiment. Care was taken not to systematically allocate the stationary, easiest to handle, beetles from each group of five to the same experimental groups. Similarly, beetles were randomly allocated between groups with respect to emergence order.

There is greater variation in the sizes of adults emerging later from a culture box (pers. obs.). To minimise the effects of this phenomenon in experiments which involved the regular replacement of accompanying beetles, daily sub-populations were set up during the previous month and virgin beetles were taken from the sub-population with the most recently emerged adults.

2.2.2. Measuring age and longevity

The age of a beetle was measured from its time of emergence. This was estimated as the time mid-way between the time at which emerging adults were last removed from the cell trays and the time at which the last beetle was removed at this inspection.

There are two problems with this measurement. First, beetles do not emerge at a constant rate; far more beetles emerge from 0800 to 2200 than from 2200 to 0800 (pers. obs.). Second, because it takes an appreciable amount of time to remove beetles from the cell-trays, there may be slight differences in the time periods over which the beetles removed during a single inspection period have emerged. To avoid the problems associated with such factors, beetles were allocated randomly to experimental groups with respect to order of removal from the cell-trays.

When determining longevity, beetles were checked at 12 h intervals to determine whether or not they were dead. The longevity of a beetle was calculated by subtracting the mid-time of emergence from an estimate for the time of death, taken as the mid-point of the interval during which death occurred. This estimate for the time of death depends on beetles being equally likely to die at any time during each 12 h period.

2.2.3. Measuring reproductive output

The number of eggs laid by a female may be constrained by the provision of too few beans (Credland, 1986). Ovenden (1991) found, using this strain of *C. maculatus*, that there was no difference in the numbers of eggs laid by females provided with a total of either 25 or 70 beans. The benefits of reducing the number of beans available to females, in terms of hours counting, are considerable. These benefits vary with the size of the experiment. The minimum provisioning of beans was either 5 beans per day or 20 beans in total.

There are several developmental stages through which offspring pass before emerging as adults. Between oviposition and the development of the first instar larva, the egg is translucent with no discernible internal structure. The first instar larva is distinguished from an undifferentiated egg by its dark cephalic shield (van de Meer, 1979) which is visible through the egg membranes. The next recognisable developmental stage is when the larva hatches from the egg and burrows through the testa into the cotyledon: either the egg turns white as the chorion is filled with material displaced by the larva, or a small hole is visible.

The stage at which offspring were counted depended on the aspect of reproductive output which was being studied. Maternal investment in offspring was measured as the total number of eggs which were laid, irrespective of their subsequent development. Ideally it should have included the number of eggs remaining in the reproductive tract after death. Because of the impracticality of counting these eggs in each female, these eggs were ignored. This is partly justifiable because the number of eggs remaining in the reproductive tract after death is small, 0 - 6 (Bonser, cited by Ovenden, 1991) and, because they are partially resorbed, they represent less investment than oviposited eggs (Wilson, 1989). Fertility was measured as the number of fertile eggs laid (the methods are described in detail in Chapter 5). In addition to those eggs which develop normally this measurement included eggs which developed only to the first instar. It was impractical to distinguish between infertile eggs and individuals which died before reaching the first instar. Fecundity was estimated as the number of eggs laid including infertile eggs.

2.2.4. Measuring the lengths of eggs and elytra

Eggs, still attached to beans, were measured non-destructively using a Kontron Videoplan Image Analysis System (Kontron 8057 Eching, Munchen, Germany) linked to a Reichert Jung Polyvar optical microscope (Reichert AG, Wien, Austria). The problems associated with measuring egg size are discussed in detail by Ovenden (1991). In summary, he found that no other index of egg size based on area or volumetric parameters was a better correlate of egg weight than egg length and that measurement using an image analyser was better, in terms of measurement error, than using an eyepiece graticule installed in a conventional microscope.

The length of the right elytron was routinely measured as an index of body size using the image analyser. It was removed from the beetle after death and placed, unattached on a microscope slide. If the right elytron was damaged, the left was used. There is no evidence of directional asymmetry in elytral length (M. Sullivan, unpubl. results).

2.2.5. Weighing eggs and adults

i. Weighing eggs

Eggs were removed from the bean by distorting the testa with a mounted needle and prising the egg from the bean using a scalpel. It was difficult to remove fresh eggs without rupturing them; when dried eggs were required for analytical purposes, they could be removed far more quickly if they were partially dried while still attached to the bean. Batches of eggs in pre-weighed aluminium foil cups were weighed to the nearest μg using a Cahn 29 electrobalance (Cahn Instruments, Cerritos, CA, USA).

ii. Weighing adults

Adult beetles were weighed individually, also using the Cahn 29 electrobalance. Live adult beetles were anaesthetised by exposure to carbon dioxide for 30 s. The carbon dioxide was warmed, using a heat exchanger in a water bath. This prevented both cooling of the beetles and condensation of atmospheric water on the beetles which would have affected the weight

measurement. The weights of live beetles declined continuously on the weighing pan; a reading was taken after 30 s. Anaesthetising adults with carbon dioxide inevitably influences aspects of their behaviour and physiology (Nicholas and Sillans, 1989). In most instances, elytral length after death was used as an index of body size; the innaccuracy of the measurement was tolerated to avoid carbon dioxide influenced results.

The residual dry body weight of adults once they had died was measured by drying them to a constant weight in an oven at 60 ^{o}C and then weighing them individually on the electrobalance.

2.3. Statistical methods

Data was processed primarily on the University of Sheffield's IBM 3083 computer. Statistical analysis was performed using SPSS (SPSS Inc. 1989) or SAS (SAS Institute Inc. 1985). Purpose written BASIC programs were run on a BBC microcomputer, for randomly allocating large numbers of beetles to experimental groups. Statistical methods were obtained from Sokal and Rohlf (1981), Snedecor and Cochran (1967) and Siegel and Castellan (1988).

3. The cost of reproduction for females

3.1. Introduction

This chapter investigates the trade-off between current and future reproduction, the 'cost of reproduction' (Williams, 1966), in female *C. maculatus*. The relationship between lifetime fecundity and adult longevity was investigated using (a) phenotypic correlations, (b) partial phenotypic correlations controlling for variation in resource availability, and (c) between group regressions following experimental manipulation of lifetime fecundity.

3.1.1. Previous measurements of the cost of reproduction

A phenotypic approach has been used to measure the cost of reproduction for females in many taxa. Often, a cost of reproduction is inferred if increased reproduction results in reduced longevity. However, the cost of reproduction is defined as the trade-off between current and future reproduction (Williams, 1966); reduced longevity is only relevant to the cost of reproduction if it reflects reduced reproduction. No attempt is made to review the studies purporting to measure the cost of reproduction in depth here; this has been done elsewhere (Bell and Koufopanou, 1986; Partridge, 1989; Dijkstra *et al.* 1990, Lindén and Møller, 1991; Lessells, 1991). However, the general results of these studies are summarised below.

Phenotypic correlations have been used to demonstrate trade-offs, often where experimental manipulations are impractical for methodological or ethical reasons. For example, female northern elephant seals *Mirounga angustirostris* that were primiparous at age 3 years, had a lower survivorship for each of the next 5 years than females which deferred pupping until age 4 years (Reiter and Le Boeuf, 1991). This cost was observed in spite of the fact that the females which pupped earlier had been in superior condition when they were weaned. However, this finding that females which invested more heavily in reproduction were in superior condition is often used to explain the absence of negative phenotypic

correlations (*e.g.* Richardson's ground squirrels *Spermophilus richardsonii*: Michener and Locklear, 1991; natterjack toad *Bufo calamita*: Tejedo, 1992).

Experimental manipulations control for genetic or phenotypic differences between females by randomly allocating them between experimental groups. Therefore, in contrast to phenotypic correlations, there are no grounds to dismiss positive correlations between traits as invalid evidence for the lack of a trade-off. The cost of reproduction has consistently been demonstrated using experimental manipulations (Lessells, 1991). For example, direct manipulation of clutch size in the collared flycatcher *Ficedula albicollis* showed that parents with enlarged clutches had a reduced subsequent fecundity (Gustafsson and Sutherland, 1988). In many studies, manipulations are less direct. For example, the reproductive rate of the waterstrider *Gerris buenoi* has been manipulated by controlling food levels (Rowe and Scudder, 1988); reduction in food level resulted in a reduction in female reproductive rate and an increase in longevity. Rowe and Scudder (1988) argued that this increase in longevity was caused by the reduction in reproductive rate and was evidence of a cost of reproduction. An alternative explanation is that the manipulation affected longevity independently of its effect on reproduction. Manipulations which affect fecundity more directly, such as egg removal or restriction of oviposition sites, make the argument that the other trait was affected independently less plausible. However, the problem can never be eliminated.

3.1.2. Previous measurements of the cost of reproduction in *C. maculatus*

The cost of reproduction has previously been studied in *C. maculatus* using both of the above phenotypic approaches. Phenotypic correlations between the two traits were positive (Wilson, 1989; Møller *et al.*, 1989b). However, when lifetime fecundity has been manipulated by varying both oviposition site and male availability, this manipulation led to a negative relationship between adult longevity and lifetime fecundity (El-Sawaf, 1956; Wilson, 1989; Møller *et al.*, 1989b). This negative relationship was attributed to a trade-off between the two traits (Wilson, 1989; Møller *et al.* 1989b).

Other environmental manipulations of lifetime fecundity using temperature or nutritional manipulations resulted in positive and negative correlations respectively (Møller *et al.*, 1989b). These manipulations were not considered to measure the trade-off. It was hypothesised that temperature and nutritional manipulation created new environments and altered options sets (Møller *et al.*, 1989b; Smith, 1991); the extent to which adult longevity was affected by lifetime fecundity and the extent to which it was directly affected by the manipulation itself was unknown.

C. maculatus is one of the few species for which attempts have been made to demonstrate the trade-off between lifetime fecundity and adult longevity using genetic correlations as well as experimental manipulations. The uncorrected genetic correlation between these two traits was positive (Møller *et al.* 1989a). However, there were positive genetic correlations between both traits and developmental rate (developmental rate being the reciprocal of developmental period, the time between oviposition and emergence). These genetic correlations demonstrated a genetically based variation in the resources available to adults, because developmental rate influences the size attained by the adult. A partial correlation, controlling for the effects of weight differences, was calculated in an attempt to control for this genetic variation. This corrected genetic correlation between 'residual' adult longevity and 'residual' lifetime fecundity was not significantly different from zero. Thus, assuming that size at emergence is the sole mutual genetic cause of the positive correlations between both traits and developmental rate, this genetic correlation is evidence that there is no genetic basis to the trade-off between adult longevity and lifetime fecundity in *C. maculatus*,

3.1.3. Aims

The aims of this chapter were:

1. To contrast experimental manipulations with phenotypic correlations as a method of demonstrating the cost of reproduction.

2. The extent to which experimental manipulations had independent effects on adult longevity and lifetime fecundity was assessed.

3. The extent to which reduced longevity reflects reduced potential for future reproduction was assessed.

3.2. Methods

3.2.1. Experiment 1: Phenotypic correlation and partial correlation controlling for size at emergence

Virgin beetles 22-26 h after emergence were weighed to the nearest μg. Each female was then placed in a 25 ml container with 2 virgin males of the same age and approximately 50 black-eyed beans. Beetles were checked at 12 h intervals to determine their longevity. Once the female had died her elytral length was measured and her lifetime fecundity, the number of eggs laid on beans and the container, was scored.

3.2.2. Experiment 2: Experimental manipulations of lifetime fecundity

Two experimental manipulations of lifetime fecundity were carried out. In the first (Experiment 2a) the effects on adult longevity of manipulating both the availability of oviposition sites and males were investigated. A second experiment was needed to compare the measured trade-off (Experiment 2b) with the patterns of resource allocation measured in females sampled from the same population (resource allocation in these females will be measured and compared with this trade-off in Chapter 4). Measurement of the trade-off was made more precise in this second experimental manipulation by measuring the time of female emergence more accurately. Furthermore, in the light of the results from the first experiment, male availability was not manipulated.

a. Manipulation of the availability of both oviposition sites and males

Virgin females, 15-25 h after emergence, were randomly allocated to one of the following groups which differed in the availability of both oviposition sites and males:

Group 1: female + male + 5 whole beans

Group 2: female + male (removed after 12 h) + 5 whole beans

Group 3: female + male + 5 half beans

Group 4: female + male + 5 half beans with their seed coat removed

Group 5: female + male

Group 6: female + male (removed after 12 h)

The beetles and beans were placed in 25 ml containers and females were checked at 12 h intervals to determine their longevity. Beans were replaced with similar fresh beans every 24 h. Lifetime fecundity, including eggs laid on beans and container, and the female's elytral length were measured after the female's death.

b. Manipulation of oviposition sites with precise measurement of female time of emergence

Virgin females, sampled in successive 2 h periods, were randomly allocated to one of the following groups which differed in the availability of oviposition sites:

Group 1: 5 whole beans

Group 2: 5 half beans

Group 3: 5 half beans with their seed coat removed

Group 4: no beans

The beetles and beans were placed in 25 ml containers with a virgin male which was removed after 24 h. Females were checked at 12 h intervals to determine their longevity. Beans were replaced with similar fresh beans every 24 h. Lifetime fecundity, including eggs laid on beans and container were measured after the female's death.

3.2.3. Experiment 3: Investigation of the direct effect of oviposition site availability on adult longevity

The possibility that restriction of oviposition sites had a direct effect on adult longevity in addition to any effect caused by its effect on fecundity was investigated. Virgin females, 1-14 h after emergence, were randomly placed in 25 ml containers, without males, with either 5 whole beans, 5 half beans or without beans. They were checked at 12 h intervals to determine their longevity. Their lifetime fecundity, including eggs laid on beans and container, was recorded after their death.

3.2.4. Experiment 4: Investigation of the effect of increased longevity on future reproduction

The reproductive importance of increased longevity was assessed by randomly allocating virgin females, 1 - 7 h after emergence, to one of three groups. Females were each placed in a 25 ml container with a virgin male. Groups differed in the availability of black-eyed beans: each female in group 1 was given 5 fresh beans daily for 7 d, females in groups 2 and 3 were given 5 fresh beans daily for 7 d after being kept for 2 and 4 d respectively without beans. Their daily fecundities were recorded.

3.3. Results

3.3.1. The phenotypic correlation between longevity and fecundity

i. Data from Experiment 1

Females had a mean lifetime fecundity of 91.0 eggs (s.d. = 16.6 eggs, n = 142). Their mean longevity was 7.21 d after being placed with beans (s.d. = 1.76 days, n = 142). Both adult longevity and lifetime fecundity were positively correlated with weight at emergence (Fig. 3.1). Longevity was more strongly correlated with emergence weight than with elytral length

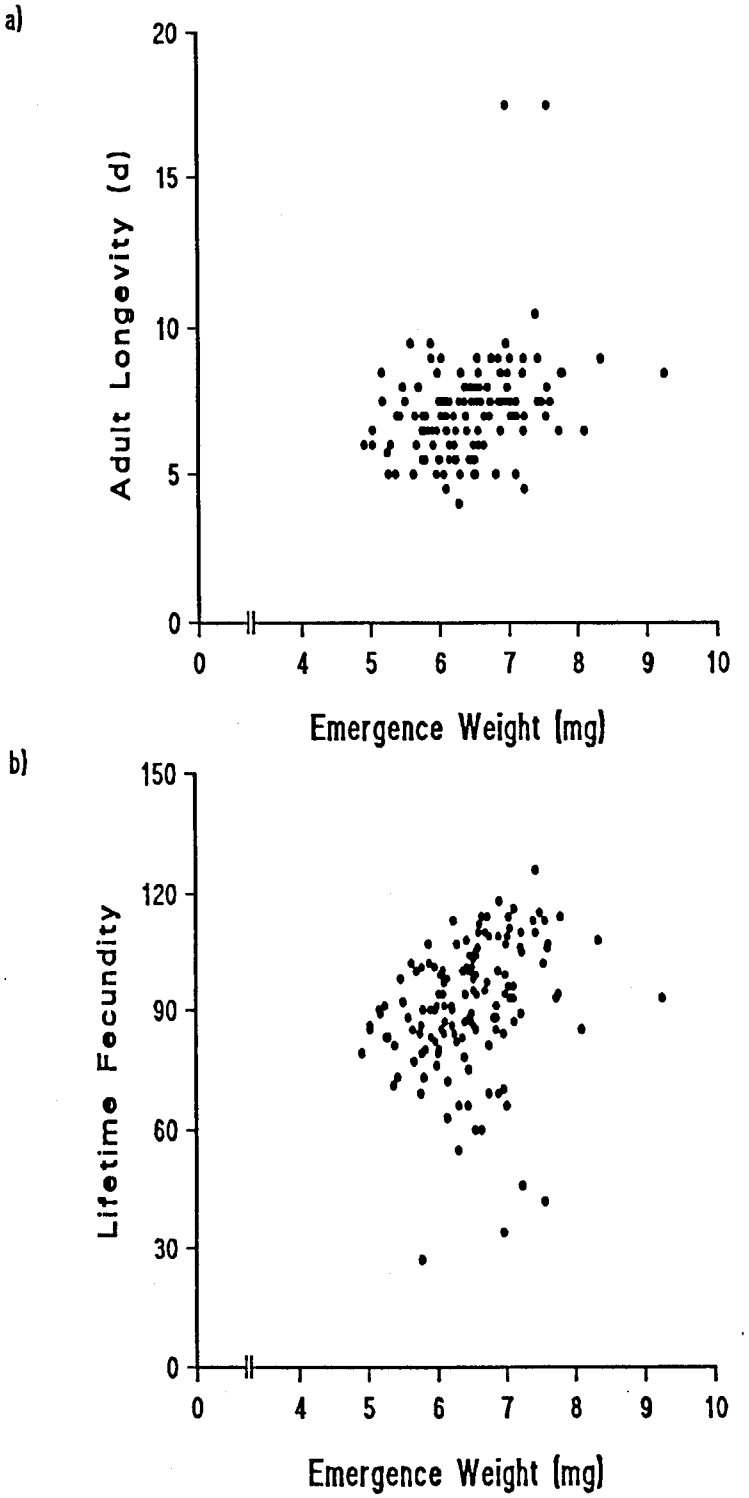

Fig. 3.1 Relationships for female C. *maculatus*. a) between longevity and emergence weight (Spearman rank correlation: $r_s = 0.355$, n = 142, $p < 0.001$; Pearson correlation: r = 0.321, n = 142, $p < 0.001$), and b) between lifetime fecundity and emergence weight ($r_s = 0.378$, n = 142, $p < 0.001$; r = 0.260, n = 142, $p < 0.01$). Females were placed with 2 virgin males and 50 black-eyed beans.

(Table 3.1). There was a positive phenotypic correlation between adult longevity and lifetime fecundity (Fig. 3.2).

ii. Data from Experiments 2a and 2b

Data from the experimental manipulations could also be used to calculate the phenotypic correlation between fecundity and adult longevity. There were significant negative correlations for several groups (Table 3.2); no groups showed positive correlations. When data for each of Experiments 2a and 2b were standardised (by subtraction of the relevant group mean followed by division by the relevant group s.d.) and combined across groups they both showed a significant negative correlation (Fig. 3.3a,b).

3.3.2. Controlling statistically for variation in resource availability (Experiment 1)

In an attempt to reduce the confounding effects of resource level variation between females, the partial correlation between adult longevity and lifetime fecundity was calculated, controlling for emergence weight. Emergence weight rather than elytral length was used to indicate resource availability because it was more strongly correlated with both fecundity and longevity (Table 3.1). Adult longevity and lifetime fecundity were not correlated after controlling for emergence weight (partial correlation coefficient: $r = 0.148$, $p > 0.05$). One problem with this analysis is that the data were not normally distributed. When outliers (determined by eye and indicated in Fig. 3.2) were removed, there was a positive partial correlation between the two traits ($r = 0.248$, $p < 0.05$).

3.3.3. Experimental manipulation of fecundity (Experiment 2)

i. Experiment 2a

The lifetime fecundities of females were affected by the way in which their group was manipulated. Consequently, each group had a different mean lifetime fecundity. Group mean

Table 3.1 Correlations between female traits measured in Experiment 1.

Spearman rank correlations between pairs of traits measured in 142 females from Experiment 1. Levels of significance in brackets.

	Emergence weight	Elytral length	Adult longevity
Elytral length	0.842 (< 0.001)		
Adult longevity	0.355 (< 0.001)	0.227 (< 0.01)	
Lifetime fecundity	0.378 (< 0.001)	0.333 (< 0.001)	0.313 (< 0.001)

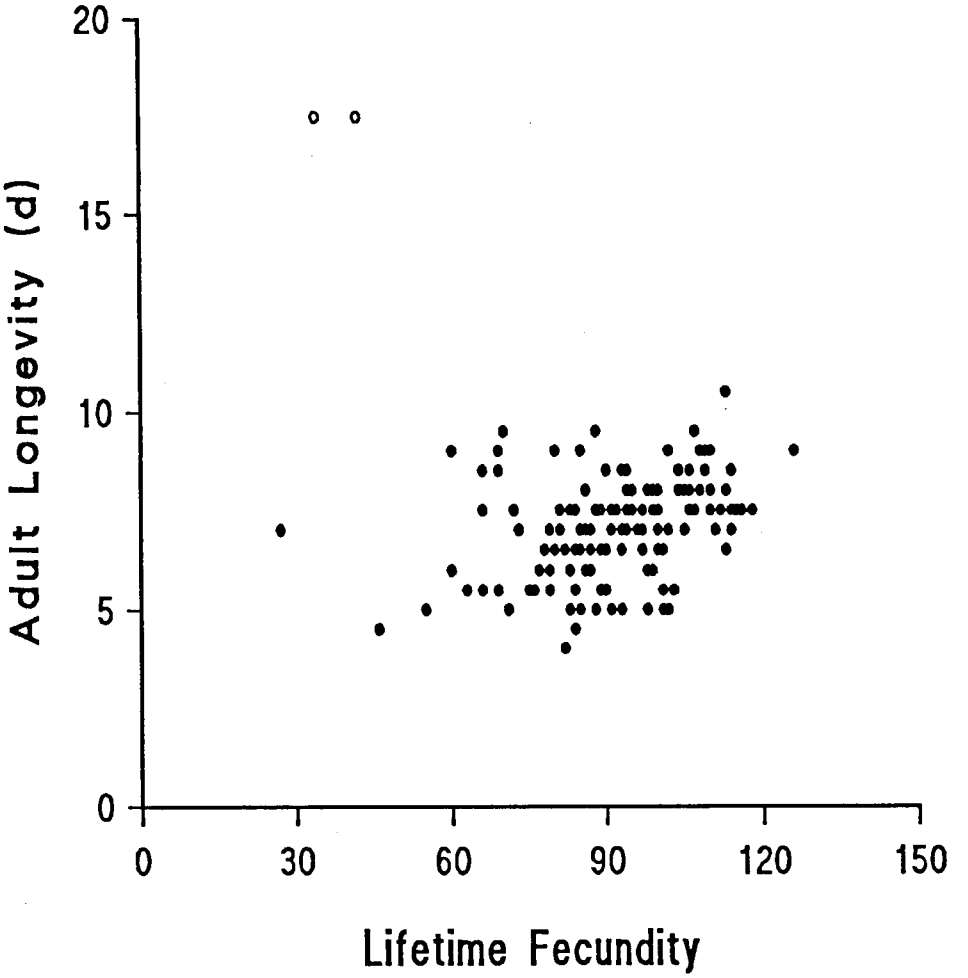

Fig. 3.2 The phenotypic correlation between longevity and lifetime fecundity for *C. maculatus*. (Spearman rank correlation: $r_s = 0.313$, $n = 142$, $p < 0.001$; Pearson correlation: $r = -0.052$, $n = 142$, $p > 0.05$). Outliers removed in some analyses (see text) are indicated by open circles. Females were placed with 2 virgin males and 50 black-eyed beans.

Table 3.2 Spearman rank correlations between lifetime fecundity and adult longevity within each group of females from Experiments 2a and 2b (see 3.2.2 for an explanation of group numbers).

	r_s	n	p
Experiment 2a			
Group 1	- 0.429	14	> 0.05
Group 2	- 0.297	15	> 0.05
Group 3	- 0.694	15	< 0.01
Group 4	- 0.117	15	> 0.05
Group 5	- 0.510	15	> 0.05
Group 6	- 0.473	15	> 0.05
Experiment 2b: replicate 1			
Group 1	0.155	20	> 0.05
Group 2	- 0.456	20	< 0.05
Group 3	- 0.591	20	< 0.01
Group 4	- 0.123	20	> 0.05
Experiment 2b: replicate 2			
Group 1	0.329	20	> 0.05
Group 2	- 0.696	20	< 0.001
Group 3	0.085	20	> 0.05
Group 4	- 0.315	20	> 0.05

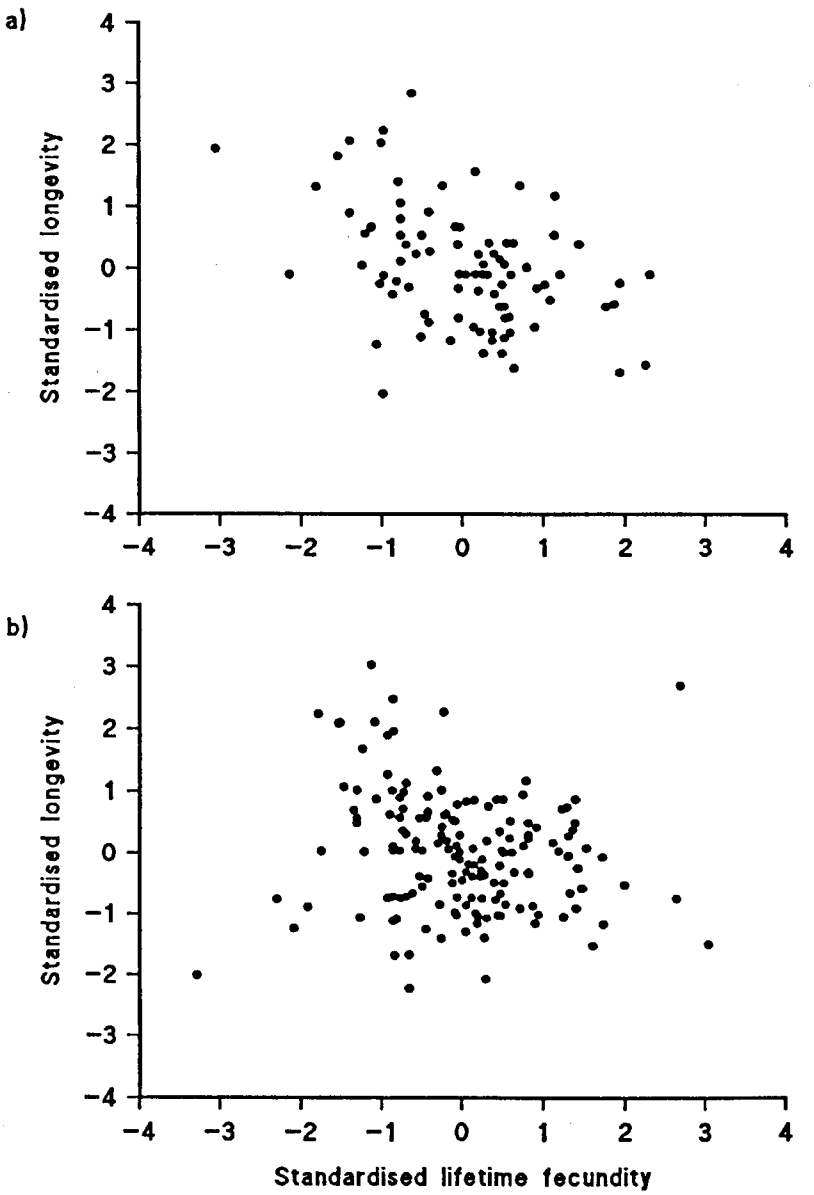

Fig. 3.3 The relationship between longevity and fecundity in female *C. maculatus*. Data are from a) Experiment 2a, Spearman rank correlation: $r_s = -0.397$, $n = 59$, $p < 0.005$; b) Experiment 2b, Spearman rank correlation: $r_s = -0.397$, $n = 59$, $p < 0.005$. Values have been standardised by subtracting the relevant treatment means from the values for individual females and dividing by the relevant group s.d.

lifetime fecundities were between 20.5 and 96.7 eggs and group mean adult longevities between 7.7 and 16.6 d. Groups did not differ in mean elytral lengths ($F_{5, 82} = 0.53$, $p > 0.05$) and, because elytral length and emergence weight are strongly correlated (Table 3.1), were unlikely to differ in mean emergence weights. This confirmed that females were allocated randomly between groups with respect to resource availabilities. There was a negative relationship between adult longevity and lifetime fecundity, irrespective of whether analysis included those groups in which males were removed (Fig. 3.4).

Removal of males, in contrast to the restriction of oviposition site availability, affected female longevity independently of its effect on lifetime fecundity. Removal of males after 12 h had little effect on lifetime fecundity (comparisons between groups 1 *vs.* 2, and 5 *vs.* 6: t-tests, $p > 0.05$) but resulted in increased longevity in group 2 compared with group 1 (t-test, $p < 0.05$) (Fig. 3.4).

i. Experiment 2b

Group mean lifetime fecundities were between 10.4 and 104.6 eggs and group mean adult longevities between 8.8 and 21.0 d. Regressions of treatment means did not differ significantly between replicates (Table 3.3). When the data were combined there was a significant negative relationship between adult longevity and lifetime fecundity (Fig. 3.5).

3.3.4. The direct effect of oviposition site availability on adult longevity (Experiment 3)

Manipulation of oviposition site availability had no effect on the longevity of virgin females; the three groups of virgin females with manipulated oviposition site availability differed in neither their mean lifetime fecundity ($F_{2, 52} = 1.34$, $p > 0.05$) nor mean adult longevity ($F_{2, 52} = 0.66$, $p > 0.05$). This suggests that any effect on the longevity of reproducing females is a consequence of their manipulated fecundity.

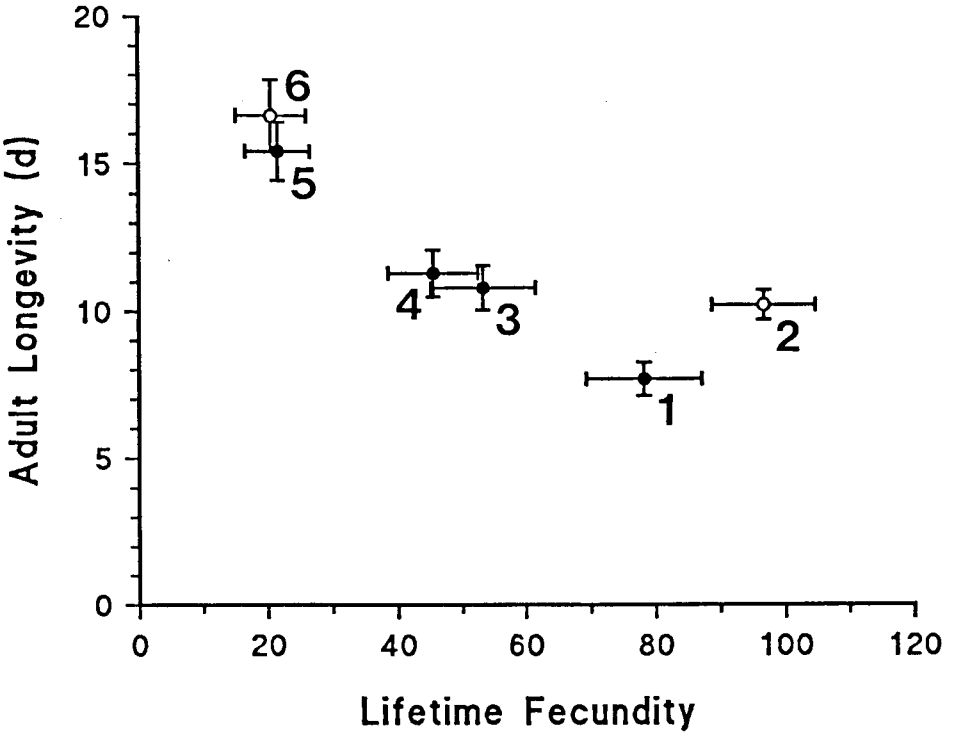

Fig. 3.4 The relationship between longevity and lifetime fecundity for experimentally manipulated groups of females (Experiment 2a). Groups differed in the availability of oviposition sites and males. Numbers refer to groups: 1. female, male and 5 whole beans; 2. female, male and 5 whole beans (male removed after 12 h); 3. female, male and 5 half beans; 4. female, male and 5 half beans with their seed coat removed; 5. female and male; 6. female and male (male removed after 12 h). Error bars show the standard errors of the means. Regression of treatment means: longevity (d) = 22.8 - 0.365 fecundity + 0.00237 fecundity^2. $F_{2,3} = 33.6$, $p < 0.01$. The fitted curve was significantly non-linear: $F_{1,3} = 1.91$, $p < 0.05$. When groups 2 and 6 (those in which males were removed after 12 h) were excluded from the analysis: longevity (d) = 18.5 - 0.149 fecundity. $F_{1,3} = 230.1$, $p < 0.01$.

Table 3.3 Comparison of the slopes and elevations of the observed trade-offs between adult longevity and lifetime fecundity of female *C. maculatus* from replicates 1 and 2 (see 3.2.2). F values are for a two-tailed F-test checking for homogeneity of variances (Snedecor and Cochran, 1967, pp. 46-47) and for a comparison of slopes and elevations (Snedecor and Cochran, 1967, pp. 432-436).

	F	d.f.	p
Comparison of variances	11.7	2, 2	> 0.05
Comparison of slopes	1.40	1, 4	> 0.05
Comparison of elevations	0.29	1, 5	> 0.05

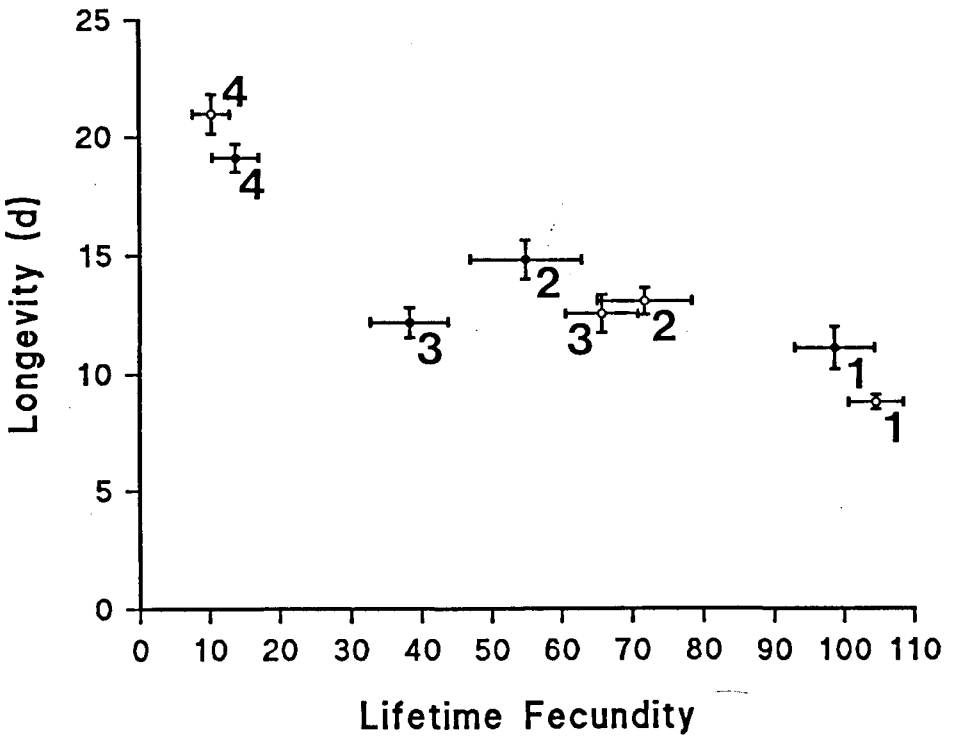

Fig. 3.5 The relationship between longevity and lifetime fecundity for experimentally manipulated groups of female *C. maculatus* (Experiment 2b). Groups differed in the availability of oviposition sites. Numbers refer to groups: 1. female and 5 whole beans; 2. female and 5 half beans; 3. female and 5 half beans with their seed coat removed; 4. female with no beans. Error bars show the standard errors of the means. Open and closed circles refer to replicates 1 and 2 respectively (see text). There were no differences between replicates in the regressions of treatment means (see Table 3.3) so the data were combined. Regression of treatment means: longevity (d) = 20.08 - 0.105 fecundity. $F_{1,6} = 25.6$, $p < 0.01$.

3.3.5. The effect of increased longevity on future reproduction (Experiment 4)

The mean lifetime fecundity of females able to oviposit throughout their lives, 98.9 eggs (s.e. = 3.61), was significantly higher than those of females prevented from ovipositing for 2 or 4 d, 85.4 eggs (s.e. = 6.11) and 78.9 (s.e. = 5.39) respectively (ANOVA comparing the 3 group means: $F_{2,56} = 3.81$, $p < 0.05$). However, the later daily fecundities of females unable to oviposit early in life were consistently higher than females with less restricted access to beans (Fig. 3.6; see Table 3.4 for a numerical comparison).

3.4. Discussion

There was a negative correlation between the mean longevity and mean lifetime fecundity of groups of female *Callosobruchus maculatus* following manipulation of fecundity. The regressions suggest that each egg reduces a female's lifespan by 3.6 (Experiment 2a) and 2.5 h (Experiment 2b). This negative correlation must be due to a trade-off between lifetime fecundity and adult longevity because it is not due to independent direct effects of the manipulation of longevity. First, the negative correlation persists when groups involving the removal of males (groups 2 and 6, Experiment 2a) are excluded from the analysis, so cannot be due to a direct effect of the presence of males on female longevity (Fowler and Partridge, 1989). Second, altering the availability of oviposition sites does not exert a direct effect on adult longevity; manipulation of the availability of oviposition sites to groups of virgin females, which laid similar low numbers of infertile eggs, had no effect on their longevity (Experiment 3).

The reproductive relevance of increased adult longevity is shown by the increased later fecundity of females prevented from ovipositing early in life (Experiment 4). The trade-off between lifetime fecundity and adult longevity is therefore evidence of a trade-off between current reproduction and future reproduction, the cost of reproduction.

The relationships measured between adult longevity and lifetime fecundity do not differ from each other or from those observed in previous similar experimental manipulations of C.

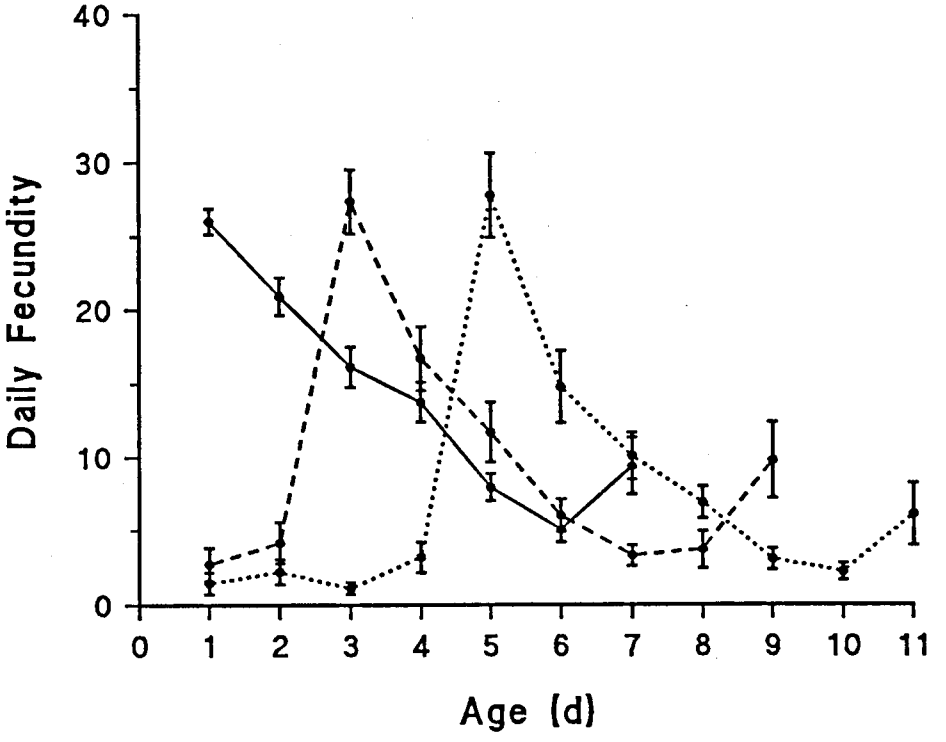

Fig. 3.6 The effect of restricting oviposition early in life on the daily fecundities of female C. *maculatus*. Females were provided with 5 fresh black-eyed beans daily for 7 d from the time of emergence (solid line), from 2 d after emergence (dashed line) or from 4 d after emergence (dotted line). Daily fecundity was recorded for the first 6 d on which beans were provided. Beans provided on the seventh day were not removed and thus the eggs recorded on these beans include eggs oviposited on the seventh day of bean provision and thereafter.

Table 3.4 Comparison of the fecundities later in life of experimentally manipulated groups of females (Experiment 4). Groups differed in the time after mating at which oviposition sites were made available (for more detailed methods see 3.2.4). Fecundities were measured from the beginning of the day indicated until the female's death. There were significant differences between groups in their later fecundities (ANOVAs comparing the fecundities of each group from the day indicated: day 5 till death, $F_{2, 56} = 41.97$, $p < 0.001$; day 6 till death, $F_{2, 56} = 27.50$, $p < 0.001$; day 7 till death, $F_{2, 56} = 14.43$, $p < 0.001$)

	Number of days without oviposition sites	Fecundity (s.e.)		
		Day 5 till death	Day 6 till death	Day 7 till death
Group 1	0	22.74 (1.48)	14.79 (1.55)	9.84 (2.01)
Group 2	2	34.50 (3.12)	22.80 (2.44)	16.80 (2.57)
Group 3	4	70.95 (5.65)	43.15 (3.79)	28.35 (2.69)

maculatus (Table 3.5). Given the inevitable environmental differences and genetic differences between populations, this indicates that the trade-off is remarkably stable.

In contrast to the experimental manipulations, the phenotypic correlations are inconsistent; one is positive and the other negative, while previous studies have reported positive phenotypic correlations (Møller *et al.* 1989a; Wilson in Lessells, 1991). Phenotypic correlations are influenced by the variance between individuals in both resource availability and allocation decisions (van Noordwijk and de Jong, 1986). If variation in resource availability is large compared with variation in allocation decisions, a positive phenotypic correlation is likely because some individuals are able to invest heavily in both traits while others can invest heavily in neither. Conversely, if there is little variation between females in resource availability compared with variation in allocation decisions, a negative phenotypic correlation is likely because individuals investing heavily in one trait must invest lightly in the other. Thus, the positive phenotypic correlations observed in this study may be due to a relatively large variation between individuals in resource availability compared with the variation in allocation decisions. The negative phenotypic correlations within groups of females in experiment 2 may be due to a relatively little variation between individuals in resource availability compared with the variation in allocation decisions. The discrepancies between different phenotypic correlations, whatever their cause, serve to underline the inadequacy of phenotypic correlations as a method of measuring trade-offs.

Attempts have been made to demonstrate trade-offs by controlling statistically for the effects of variation in resource availability on the phenotypic correlation. Both here and in the work of Møller *et al.* (1989a) controlling statistically for variation in body weight, which was strongly correlated with both traits, did not reveal a negative relationship between adult longevity and lifetime fecundity. This may have been because body weight at emergence is inadequate as a predictor of limiting resource availability, because its relationships with the two traits are more complex than the linear relationship assumed by partial correlation or because the females in these studies were laying similar numbers of eggs and there was insufficient variation in lifetime fecundity for any effect on longevity to be observed. However, this approach is fundamentally flawed. There is no way of deciding how resource

Table 3.5 Comparison of trade-off slopes measured in different studies. Regression coefficients,b, were compared using the Tukey-Kramer method (Sokal and Rohlf, 1981, pp 499-509). Standard errors are for the regression coefficient, k is the number of means from which the lines of regression were calculated and v is the degrees of freedom associated with the weighted average unexplained variance for all groups, $\overline{s}^2_{Y \cdot X}$ in the notation of Sokal and Rohlf. No pair of regression coefficients differed more than their Mean Significant Difference.

Source	b	s.e.	k	v	Difference
Wilson (1989)	- 0.1629	0.0247	4	2	n.s.
El-Sawaf (1956)	- 0.1492	0.0073	6	4	n.s.
This study (Experiment 2a)	- 0.1354	0.0123	4	2	n.s.
This study (Experiment 2b)	- 0.1045	0.0206	8	6	n.s.

availability should be controlled for. It could never be proved that a positive correlation that resulted from attempts to control for variation in resource availability was not due to inadequate attempts to control for this variation.

Møller *et al.* (1989a) found negative genetic correlations between developmental rate and both adult longevity and lifetime fecundity. They suggested that these were the cause of the positive genetic correlation found between the latter two traits. The rationale of this argument is similar to that for the existence of positive phenotypic correlations: some individuals have more resources than others, accumulated during their longer larval development and are subsequently able to invest more in both adult longevity and lifetime fecundity. It is argued that there is a positive genetic correlation between these two traits because there is genetic variation in developmental rate and hence resource availability. Therefore, genetic variance in developmental rate may itself be maintained by a trade-off between developmental rate and resource availability. Møller *et al.* (1989a) attempted to control statistically for variation in resource availability, by removing the effects of body weight using linear regression, and found no genetic correlation between the two traits. The problems of partial correlation have been discussed above, and, in addition, the assumption that developmental rate only influences longevity and fecundity through its effects on resource availability is not justifiable; there may be a host of other relevant morphological or physiological factors which are genetically linked to developmental rate. For instance, enzyme efficiency could be positively genetically correlated to both developmental rate and both lifetime fecundity and adult longevity; it need not be correlated with resource availability. Experimental manipulations provide the only means of measuring the trade-off between adult longevity and lifetime fecundity which avoid the confounding effects of these factors.

A comparison of the consistent results from experimental manipulations with the contradictory and inconsistent results from phenotypic correlations illustrates the inadequacy of phenotypic correlations as a technique for demonstrating trade-offs. The unreliability of attempting to control statistically for genetic variation in the resources available to individuals undermines the value of genetic correlations in measuring trade-offs. Experimental manipulations provide strong evidence that there is a trade-off between adult longevity and

lifetime fecundity in female *C. maculatus*. The slope of this trade-off is remarkably constant across environments.

4. Allocation of resources

4.1. Introduction

In the previous chapter a cost of reproduction in experimentally manipulated female *C. maculatus* was demonstrated; females with a high lifetime fecundity died while females with a lower lifetime fecundity were still reproductively active. In principle, this cost of reproduction could be due to two types of factors: ecological or physiological (Calow, 1979). Ecological factors, originally referred to as ethological factors (Calow, 1979), include all the risks associated with reproductive behaviours such as exposure to parasites during mating and increased danger of predation while relatively immobile during pregnancy or while caring for offspring. The physiological basis of the cost of reproduction is the competition for resources between processes contributing to present and future reproduction, where resources are defined broadly to include factors such as nutrients, energy or time.

In *C. maculatus* the cost of reproduction was demonstrated under conditions in which the ecological risks of reproduction were minimised: there was no predation risk, all females were mated once so that the consequent risk of sexually transmitted pathogens was randomised across experimental groups, and females were kept individually following mating. It is likely, therefore, that the cost of reproduction in *C. maculatus* has a physiological basis. This hypothesis is supported by two observations: provision of nutrients to females increases both their lifetime fecundity and their longevity (Møller *et al.*, 1989) and both lifetime fecundity and longevity are positively correlated with body size and hence resource levels (Wilson, 1989).

4.1.1. Estimating the cost of reproduction from measurements of resource allocation

If the cost of reproduction has a physiological basis, it ought to be possible to estimate it by measuring resource allocation. Many authors have estimated the proportion of assimilated energy which is allocated to reproduction by female invertebrates by biochemically assaying samples of females and their offspring (gastropods: 2.3 - 22%, Calow 1979; amphipods: 11%,

Dagg 1976; the rotifer *Brachionus rubens*: 55%, Pilarska 1977 calculated by Scheimer 1983; nematodes 53 - 78%, Scheimer 1983).

However, lifetime resource budgets do not necessarily reflect the cost of reproduction. This will depend, not on the proportion of resources allocated to reproduction, but on the effect that this reproductive allocation has on non-reproductive processes and hence on the probability of survival. If resource acquisition by aquatic invertebrates is constant under the conditions of the above experiments, these estimates may reflect energy denied from non-reproductive processes. If, however, reproductive allocation or resource absorption from the environment are variable, the cost of reproduction depends upon the relative timing of periods of resource absorption and reproductive allocation. To avoid this problem, authors have estimated the resources allocated to reproduction and maintainance processes during the period of maximal reproductive allocation, the breeding season. Even then, these resources may be allocated from reserves accumulated before rather than during the breeding season. The resource budget of an animal which feeds during its breeding season is too complicated to allow the cost of reproduction to be inferred from resource allocation studies. For example, growth may be either reproductive allocation if it increases the efficiency of offspring production, or non-reproductive allocation if it retards ageing (Fig. 4.1). It may also affect the efficiency of resource acquisition and hence the amount of resources available for allocation. However, estimates of the proportion of energy allocated to reproduction have been made in spite of these difficulties (*Sceloporous jarrovi*: 10 - 13%, *S. graciosus*: 19%, *Uta stansburiana*: 23 - 24%, Tinkle and Hadley, 1975).

The resource budget is simplified considerably during periods when the animal does not feed. Reproduction in some species may take place during such periods. In such animals, notably arctic breeding geese such as lesser snow geese *Anser caerulescens caerulescens* (Ankney and MacInnes, 1978) or semelparous species of fish such as coho salmon *Oncorhynchus kisutch* (Fleming and Gross, 1990), changes in body resource content reflect resource utilisation. Estimates of the proportion of energy allocated to reproduction can be made by measuring the change in body resource content and reproductive output. For example spawning coho salmon make a reproductive investment of between 5.4 and 12.7% of their somatic mass

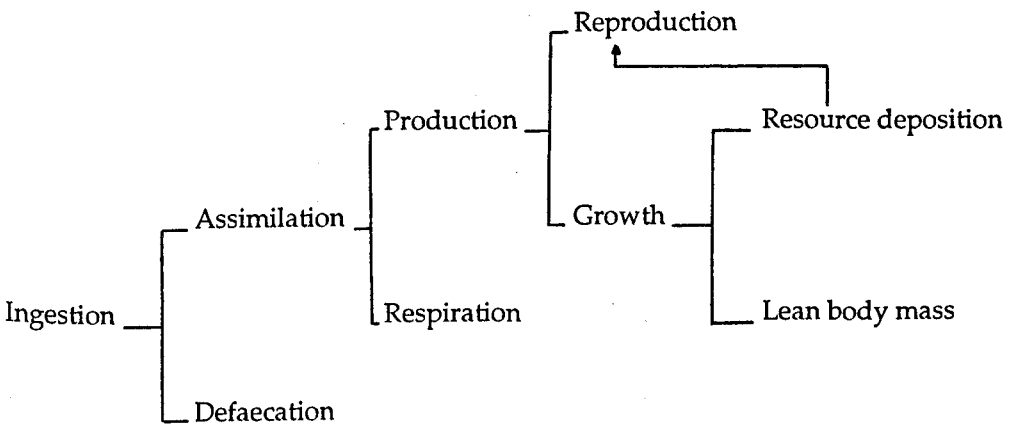

Fig. 4.1 Outline of an animal's resource budget, illustrating the metabolic pathways which must be described in order to measure the proportion of resources which are allocated to reproduction rather than to somatic maintenance (adapted from Golley, 1968; Smith, 1976).

(Fleming and Gross, 1990). In some species, resource allocation to somatic maintenance may take place during prolonged periods when the animal is not feeding, *e.g.* Allegheny Mountain salamanders *Desmognathus ochrophaeus* (Fitzpatrick, 1973). Once somatic maintenance has been measured, it may be isolated from reproductive allocation. For example, Fitzpatrick (1973) estimated that female Allegheny Mountain salamanders *Desmognathus ochrophaeus* invested 48.3% of their annual energy flow in reproductive activities. However, such estimates are more difficult to make in species where maintainance costs are confounded with elevated metabolic costs due to lactation or incubation.

To determine the cost of reproduction, the effect on survival of witholding resources from non-reproductive processes must be assessed; an overall energy budget is insufficient. The limiting resource may not be energy but nutrients such as lipid or protein, used for some specific function such as insulation or musculature. Consequently, overall energy budgets may be misleading; for example, Briegel (1990) found that there was no difference between the energetic contents of eggs laid by different sized female *Aedes aegypti*, but that the composition of these eggs varied substantially (mean protein to lipid ratios were from 1:4.3 to 1:1.3). Ankney and MacInnes (1978) have separated the lipid, protein and calcium costs of egg production and incubation for *A. c. caerulescens*. They concluded that starvation was the major cause of death during the breeding season and that the decline in body weight reflected both fat utilisation (29%) and the use of protein reserves (>29%) which were both related to the number of eggs laid and incubated.

4.1.2. Estimating the cost of reproduction in *C. maculatus* from measurements of resource allocation.

C. *maculatus* is an ideal species in which to estimate the cost of reproduction from measurements of resource allocation. Adults have no opportunity to feed; the decline in their resource content is therefore a measure of the rate of resource utilisation. They have no parental care; the resource content of their eggs is therefore a measure of a female's reproductive output. Virgin females lay a negligible number of eggs and unlaid, unfertilised

eggs are reabsorbed (Wilson, 1989). Such females may therefore be used to estimate resource allocation to non-reproductive processes. This can be compared with the resource utilisation of reproducing females to estimate the extra metabolic costs of producing eggs.

Predictions made using measurements of the allocation of different resources may be compared with an independent measurement of the trade-off between adult longevity and lifetime fecundity for the same population using experimental manipulation. The predictions made using measurements of the allocation of limiting resource should correspond with this independent estimate. However, if the estimate were very different from this independent estimate it would suggest that the measured resource was not the limiting resource.

4.1.3. Choosing suitable resources to measure

The majority of resource allocation studies have investigated energy budgets. There are two reasons for this. First, the currency of optimal foraging theory is usually energy (see Pianka, 1976). Second, there are convenient methods by which energetic budgets can be measured (*e.g.* micro-bomb calorimetry, Phillipson 1964; the doubly-labelled water technique, Lifson and McClintock, 1966).

There are two problems with investigating the budgets of other resource categories. First, unlike energy, many other resources may be created. For instance, whereas the change in an animal's energetic content during a non-feeding period would reflect its energy utilisation, the change in glucose content would not reflect its glucose utilisation; glucose may be created from other resources. This problem may be reduced by increasing the breadth of the resource category to include all interchangeable resources. However, if a resource category encompasses a large number of compounds, measurements of the allocation of this resource between processes is less likely to reflect the allocation of individual compounds.

4.1.4. Aims

1. To describe the allocation of resources between reproductive and non-reproductive processes. The resources studied were: water, lipid and energy. The allocation of dry weight

between processes was also measured. The production of water by metabolism of other resources was estimated.

2. To use these measurements to predict the slopes of the trade-off between lifetime fecundity and adult longevity that would be expected if each resource were the limiting resource and to compare these predicted trade-off slopes with the trade-off slope which was observed in females randomly sampled from the same population following experimental manipulation of lifetime fecundity.

4.2. Methods

Virgin females were used to measure both the trade-off between experimentally manipulated lifetime fecundity and adult longevity (reported in Chapter 3, summarised here) and the rate of resource allocation to processes contributing to non-reproductive processes. The allocation of dry weight, water, lipid and energy were measured. Females were sampled in successive 2 h intervals so that time of emergence was known accurately. However, successive 2 h samples of beetles are expected to differ because developmental period affects resource content (Møller *et al.*, 1990). The females were allocated randomly to all experimental groups with respect to these cohorts to avoid this problem. The experiment was replicated as a precaution against unforseen problems; dry weight and water allocation were measured for both replicates, but there were no significant differences between data from the two replicates and they have been combined in all analyses reported below.

Females, sampled on a separate occasion, were used to estimate reproductive allocation of the same resources. The resource content of their eggs was measured or, in the case of energy, estimated using published data. The total cost of egg production includes both the resource content of eggs and an additional metabolic cost. The total cost of egg production was estimated by comparing the resource contents of mated and virgin females 5 d after emergence (6 d in the case of energetic measurements). These females, originally sampled from the same population, had been randomly allocated between virgin and mated groups.

Consequently, any differences in resource content were due to the investment in eggs by the mated females.

4.2.1. Measuring the trade-off curve using experimental manipulation of lifetime fecundity

Measurement of the trade-off curve in these females has already been reported in Chapter 3. In summary, 80 virgin females were randomly allocated to groups which differed in the availability of beans and hence opportunities for oviposition. The beetles and beans were placed in 25 ml containers with a virgin male which was removed after 24 h. Females were checked at 12 h intervals to determine their longevity. After a female's death, the number of eggs she had laid during her lifetime was recorded.

4.2.2. Measuring resource allocation

i. Resource allocation to non-reproductive processes.

Virgin females were randomly allocated to all analytical samples. They were placed individually in 25 ml containers and were analysed (using the assay procedures described in 4.2.3) 1, 5, 10 or 19 d from the time of emergence or kept until death. No females died before the last sampling occasion. The longevity of females kept until death was recorded and a randomly selected sample of their cadavers was analysed for the same resources as the live beetles with the exception of free water content. Free water content was not analysed due to the probably excessive loss following death.

ii. Resource allocation to reproduction

Mated females were placed on fresh beans during their first day after emergence. Eggs were removed from these beans between 12 and 24 h later. Their resource content was measured as described in 4.2.3.

The total metabolic cost of producing eggs, including both the resource content of these eggs and the extra metabolic cost of producing eggs, was estimated by comparing the resource

content of virgin and mated females. Virgin females, removed from the same population, were individually placed in 25 ml containers, each with 20 black-eyed beans. A virgin male was placed with half of the females, selected at random, and removed after 12 h. After 5 d (6 d for energetic analysis) the virgin and mated females were sampled and their resource content was measured. The number of eggs laid by each mated female was scored. It is possible that mated females gain resources from the ejaculate. However, the evidence that mated females gain such a benefit from the ejaculate is inconclusive (see Chapters 5 and 6) and it was assumed that this source of resources was trivial.

Resource content at emergence was measured in a third group of females randomly sampled from the same population.

iii. Resource content of virgin and mated females at death

Virgin females were randomly allocated to two groups. Females in group 1 were placed in a 25 ml container with no beans or males. Females in group 2 were placed in a 25 ml container with a virgin male, which was removed after 12 h, and 20 black-eyed beans. Females were checked at 12 h intervals and dead females were stored at -70 ^{o}C. When all the females had died they were dried to constant weight at 60 ^{o}C and their resource content was analysed (see 4.2.3).

4.2.3. Assay procedures

i. Dry weight and free water content

Sampled live beetles were anaesthetised using carbon dioxide and weighed individually. They were immediately placed in a refrigerator at -70 ^{o}C to kill them. 24 h later they were placed in an oven at 60 ^{o}C, dried to constant weight and reweighed. Sampled cadavers were weighed after drying to constant weight.

Eggs were weighed in samples of ten as soon as they had been removed from the beans. They were then dried to constant weight at 60 ^{o}C and reweighed.

ii. Lipid content

A sample dry weight of at least 10 mg was necessary for total lipid analysis. Because dry weight declined with age, later analytical samples contained more females. The numbers of females which were needed each day were calculated from the results of preliminary experiments (Table 4.1).

Lipid was extracted and purified using the following methods adapted from Folch *et al.* (1957). The sample was homogenised in 400 µl chloroform:methanol (2:1 by volume). A similar volume of chloroform:methanol (1:2 by volume), used to wash the homogeniser, was added to the homogenate which was then centrifuged at 13400 g for 2 min. The solvent phase was removed and to it was added any lipid remaining in the pellet removed with two further extractions using 100 µl chloroform:methanol (1:2 by volume). The extracted sample was dried, dissolved in 200 µl Folch Lower Phase (FLP) (chloroform:methanol:water, 86:14:1 by volume) and washed with 200 µl Folch Upper Phase (FUP) (chloroform:methanol:0.74% $KCl_{(aq)}$, 3:48:47 by volume) to remove non-lipid contaminants. The FUP was washed with FLP and the combined lower phases were dried and assayed for lipid content.

The total lipid content of each sample was assayed using a method adapted from Marsh and Weinstein (1966). The lipid extract was dissolved in 200 µl chloroform. 20 µl of this solution was added to each of two thick walled test-tubes and the chloroform was allowed to evaporate. 1 ml concentrated sulphuric acid was added to each tube which were heated to 200 (± 2) °C for 15 min and then cooled in ice for 5 min. While the tubes were still in ice, 3 ml water was added. The tubes were mixed and allowed to cool. The optical density was read at 375 nm. Samples were compared with olive oil standards and a chloroform blank. Regression coefficients for the regressions of absorbance readings against olive oil standards from which these estimates were made are given in Table 4.2.

iii. Energy content

Energetic content was measured using a 1107A 22 ml Semi-micro Oxygen Bomb Calorimeter in a 1455 Solution Calorimeter (Parr Instrument Company, Illinois, USA). Adult females

Table 4.1 The number of females per sample required for lipid analysis

Preliminary experiments suggested that sample sizes with a dry weight of approximately 10 mg were needed for analysis of lipid content. The number of females per sample required for an analysis of the lipid content of virgin female *C. maculatus* was predicted using data from a preliminary investigation into the decline in virgin female dry weights with age. These data and the sample sizes used are summarised below.

Day	Number of females in each sample	Predicted dry weight of sample (mg)	No. of samples
1	3	10.77	8
5	4	11.56	8
10	5	11.95	8
15	5	10.25	8
19	5	9.95	8

Table 4.2 Regression equations for the calibration curves used to calculate the lipid content of *C. maculatus* females and their eggs (see 4.2.4 for methods). Lipid mass in sample = m.(Absorption at 375 nm, relative to blank sample containing no lipid) + c.

Experiment	m	c	r^2
Non-reproductive lipid utilisation	0.194	-1.93×10^{-3}	0.991
Egg lipid content	0.166	-4.04×10^{-3}	0.995
Extra cost of egg production	0.160	-5.54×10^{-2}	0.987
Lipid content of females at death	0.193	-70.1×10^{-6}	0.999

within each sample were randomly allocated to sub-samples each consisting of 8 beetles, with a combined dry weight of between 15 and 25 mg. Samples, dried to constant weight, were compressed to form a pellet (0.5 cm diameter) which was ignited using fuse wire (with an energetic value of 5.49 cm^{-1}) in oxygen at 30 atm.

Samples had been collected for use with another bomb calorimeter and were consequently below the recommended minimum sample size, 25 mg, for this calorimeter. However, a calibration curve measured using samples of benzoic acid with a known energetic content showed that the calorimeter was accurate well below this recommended minimum (Fig. 4.2). Samples used to measure the calibration curve resulted in changes of temperature of between 0.15 and 0.52 ^{o}C. Samples used to estimate female energetic content resulted in changes of temperature of between 0.22 and 0.47 ^{o}C, within the range for which the calorimeter had been shown to be accurate. It was decided that it would be impractical to measure the energetic content of eggs using this technique because of the huge numbers of eggs required to approach sensible sample weights. The energetic content of eggs was estimated using Wightman's (1978) data for the eggs of *C. analis*, measured using a bomb calorimeter capable of measuring the energetic content of smaller samples.

iv. Potential water content

Water, a potential limiting resource, is a by-product of many metabolic pathways. Consequently, the free water content of females at emergence is not a measure of the amount of water which is available to them during their lives. In an attempt to quantify water loss, the amount of water which could potentially be produced by metabolism of lipid and non-lipid reserves was added to the free water content to give an estimate of 'potential water content'. 'Water equivalence' factors, giving the number of g of water produced by the oxidation of 1 g of substrate, are dependent on the molecular composition of the reserves in question. For example, Brody (1945) estimated that 1 mg lipid produces 1.07 mg water; 1 mg protein produces 0.40 mg water and 1 mg carbohydrate produces 0.6 mg water. To estimate potential water content, a range of 'water equivalence' factors were used for lipid (L = 0.9 to 1.3) and

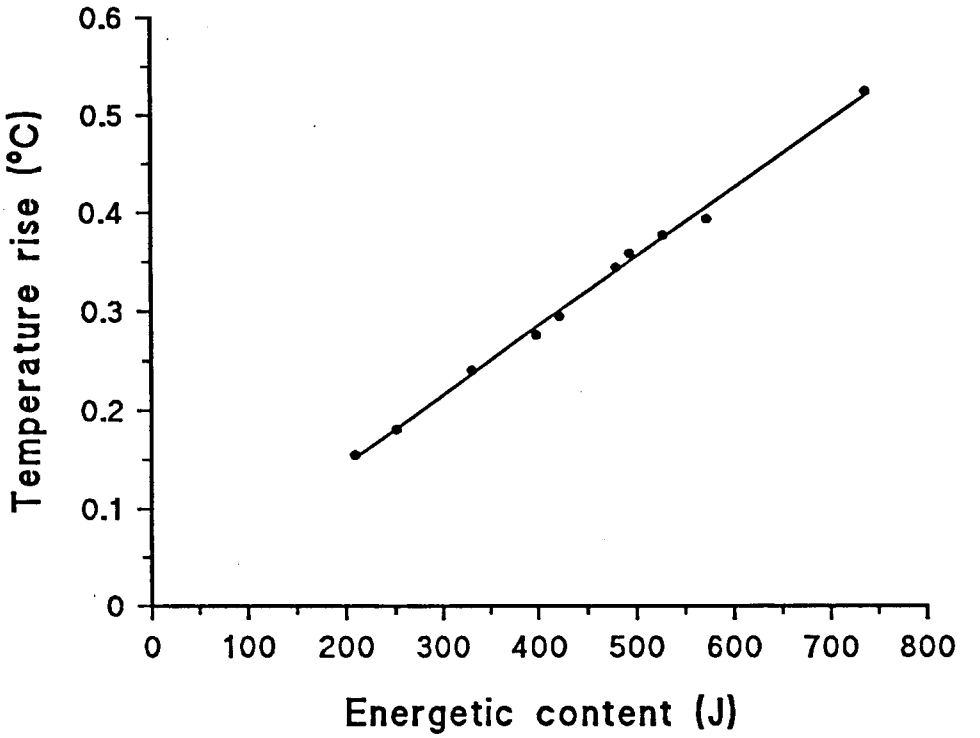

Fig. 4.2 The temperature rise recorded by the semi-micro bomb calorimeter for different quantities of benzoic acid. Samples of benzoic acid ranged from 6.86 mg to 26.52 mg. Energetic contents were calculated by adding the known energetic content of the benzoic acid sample to the energy released by the measured length of fuse wire used to ignite each sample (see 4.2.5).

non-lipid (N = 0.2 to 0.8) metabolites. These ranges of values allowed an assessment of the sensitivity of the calculations to incomplete oxidation of metabolites or oxidation of particularly hydrogen rich metabolites. The potential water content of a female or eggs was estimated as the free water content, plus the lipid and non-lipid dry weight content each multiplied by the relevant 'water equivalence' factor.

4.2.4. Calculation of errors

Standard errors are quoted for means estimated from calibration curves. The errors associated with the calibration curves have not been incorporated into these standard errors. The standard error for a mean value for a single beetle was estimated from the standard deviation of a number of samples each containing a known number of beetles:

$$s.e.(\mu) = s_Y / (p\sqrt{n})$$

where s.e.(μ) is the standard error of the mean resource content of a single beetle, s_Y is the standard deviation of the mean resource content of n samples each containing p beetles (see Appendix).

4.3. Results

4.3.1. The trade-off curve measured using experimental manipulation of lifetime fecundity

These results are reported in Chapter 3 and are summarised here. There was no significant difference between the two within replicate regressions of treatment means (Table 3.3, p.31) so a regression was calculated using the treatment means from both replicates. Treatment mean lifetime fecundities were between 10.35 and 104.6 eggs, treatment mean adult longevities between 8.8 and 21.0 d. Regression of the eight treatment means gave a negative relationship between the lifetime fecundity and adult longevity (Fig. 4.3), and the measured slope of the trade-off curve was - 0.105 (s.e. = 0.021) $d.egg^{-1}$.

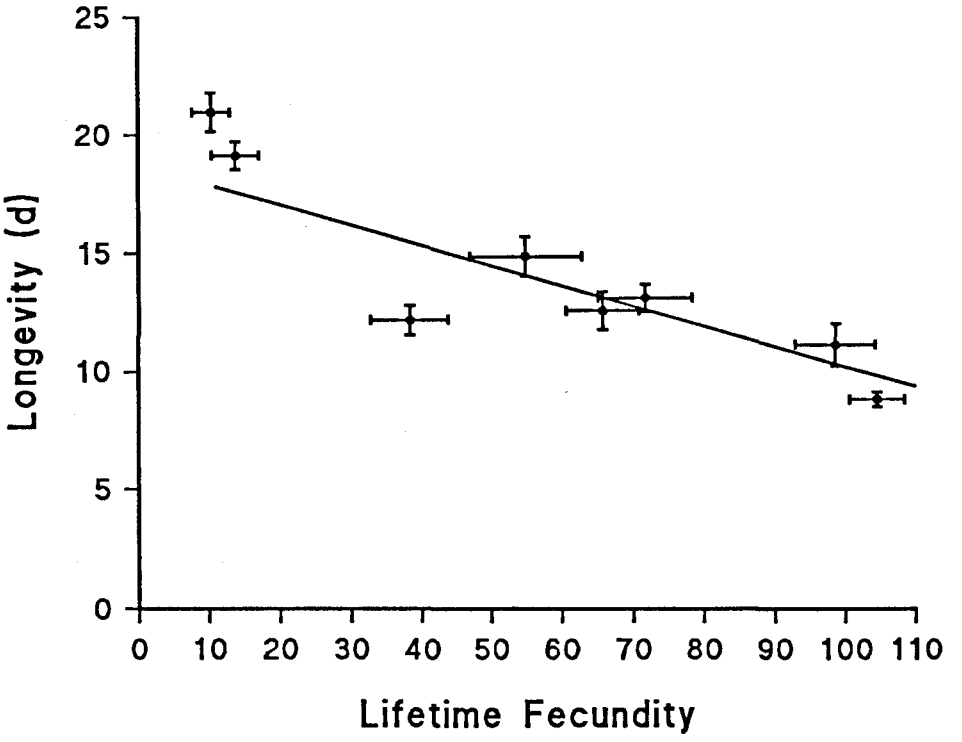

Fig. 4.3 The measured trade-off between adult longevity and lifetime fecundity for experimentally manipulated groups of females (reported in 3.2.2b). Points are treatment means (+/- standard errors). Regression of treatment means: longevity (d) = 20.08 - 0.105 fecundity. $F_{1,6} = 25.6$, $p < 0.01$.

4.3.2. Resource allocation to non-reproductive processes

There were no significant differences between replicates in the mean fresh and dry weights of virgin females sampled from 1 to 19 d after emergence (Fig. 4.4); these data were combined in subsequent analyses. The mean resource contents of virgin females sampled at differing ages and the resource contents of the cadavers of virgin females kept until death are given in Figs. 4.5a,b and Table 4.3.

From the regressions of fresh weight, dry weight and free water content against time after emergence (Fig. 4.4), the resource contents at emergence were estimated as: fresh weight 6.85 mg, dry weight 3.58 mg and free water content 3.46 mg. Free water therefore accounted for 50.5% of a female's fresh weight at emergence. From the quadratic regression of lipid content against time after emergence (Fig. 4.6), the mean lipid content of females at emergence was estimated as 1.396 mg. Thus, lipid accounted for 38.7% of a female's dry weight at emergence. The mean energetic content of females at emergence was estimated as 99.6 J, using the quadratic regression of energetic content against time after emergence (Fig. 4.7), an energetic content of 27.8 J.mg (dry weight)$^{-1}$.

Because virgin *C. maculatus* lay a negligible number of eggs, resorb unlaid eggs (Wilson, 1989) and are unable to feed as adults, their resource content could be used to estimate the rate at which each resource was allocated to non-reproductive processes. These rates were estimated as the slopes of the linear regressions between female resource content and age (Figs. 4.4, 4.6 and 4.7). This assumed that the rate of resource allocation to non-reproductive processes was constant with respect to time since emergence. The rates of allocation of each resource to non-reproductive processes are given in Table 4.4.

Virgin females kept until death had a mean adult longevity of 20.23 d (s.e. = 0.37). The mean dry weight of these cadavers was 47.3% of the estimated dry weight of a virgin female at emergence. Their mean lipid content accounted for 15.3% of their dry weight at death, 18.6% of the estimated lipid content of a virgin female at emergence. Their mean energetic content at death was 25.0 Jmg^{-1}, 42.5% of the estimated energetic content of a virgin female at

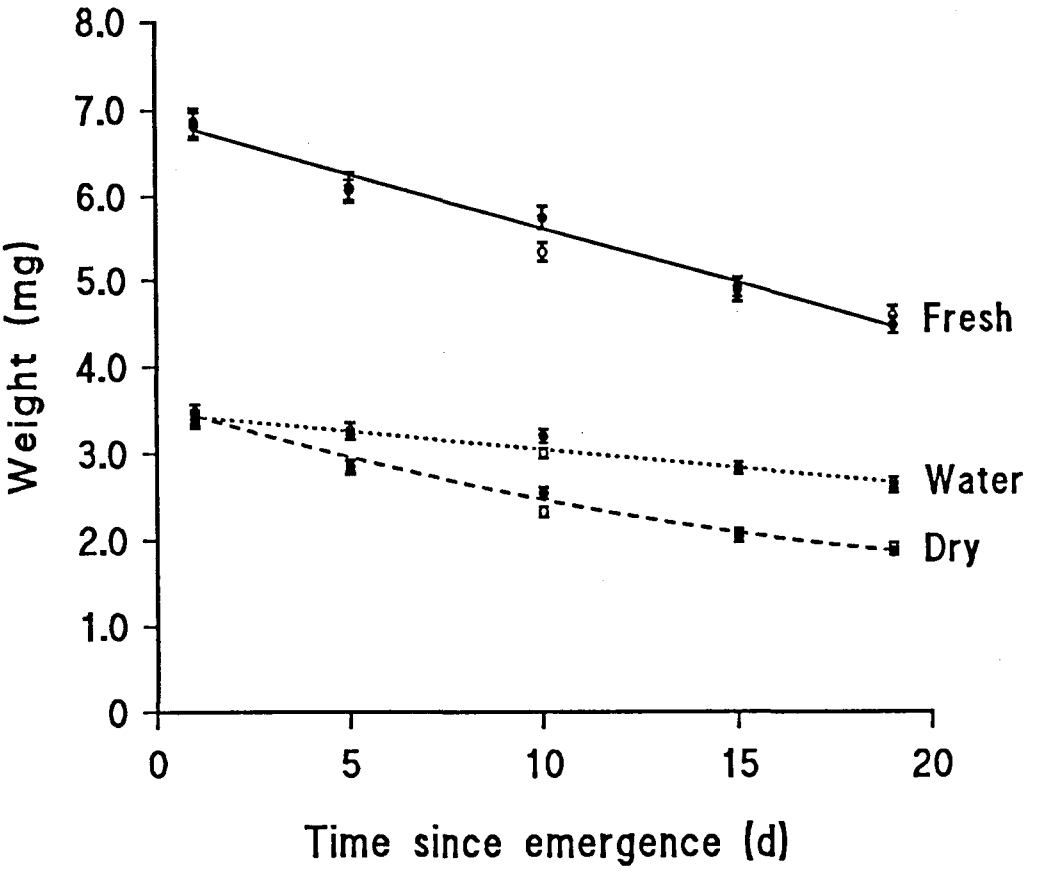

Fig. 4.4 The fresh and dry weights of virgin females. Points are means (+/- standard errors). Solid and closed circles refer to replicates 1 and 2 respectively (see 4.2). Regressions of means for the combined data:

Fresh weight (mg) = 6.85 - 0.127 t. $F_{1,3} = 251.2$, $p < 0.001$ (solid line).

Mass of water (mg) = 3.47 - 0.0423 t. $F_{1,3} = 191.99$, $p < 0.001$ (dotted line).

Dry weight (mg) = 3.58 - 0.151 t + 0.00332 t^2. $F_{2,2} = 330.5$, $p < 0.01$ (dashed line).

The fitted curve was significantly non-linear: $F_{1,2} = 24.7$, $p < 0.05$. A linear regression was used for analytical purposes (see 4.4.1): Dry weight = 3.39 - 0.0848 t. $F_{1,3} = 71.47$, $p < 0.01$.

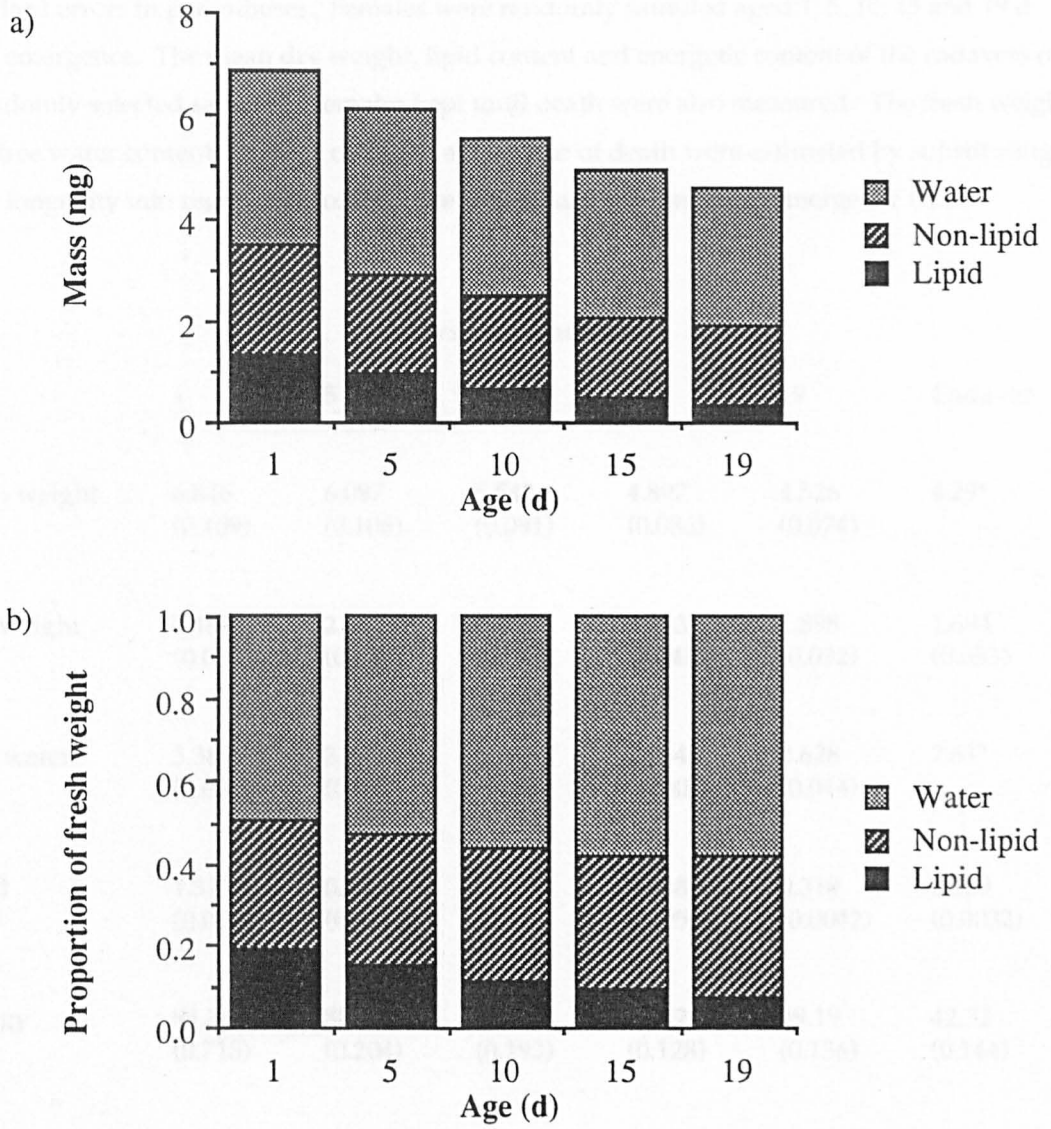

Fig. 4.5 The resource contents of virgin females sampled at intervals after emergence (data are summarised in Table 4.3). The resource contents of females are expressed as a) mass per individual and b) proportion of fresh weight.

Table 4.3 The decline in the resource contents of virgin females. Values are means with standard errors in parentheses. Females were randomly sampled aged 1, 5, 10, 15 and 19 d after emergence. The mean dry weight, lipid content and energetic content of the cadavers of a randomly selected sample of females kept until death were also measured. The fresh weight and free water contents of these cadavers at the time of death were estimated by substituting their longevity into regressions of resource content against time after emergence (*).

	Days after emergence					
	1	5	10	15	19	Cadaver
Fresh weight (mg)	6.846 (0.109)	6.097 (0.106)	5.542 (0.091)	4.897 (0.086)	4.526 (0.074)	4.29*
Dry weight (mg)	3.457 (0.057)	2.854 (0.052)	2.436 (0.045)	2.063 (0.041)	1.898 (0.032)	1.694 (0.033)
Free water (mg)	3.389 (0.056)	3.243 (0.057)	3.106 (0.049)	2.834 (0.048)	2.628 (0.044)	2.61*
Lipid (mg)	1.313 (0.021)	0.933 (0.017)	0.629 (0.010)	0.448 0.0054)	0.319 (0.0042)	0.259 (0.0032)
Energy (J)	94.36 (0.718)	80.49 (0.204)	61.38 (0.193)	54.72 (0.128)	49.19 (0.136)	42.32 (0.144)

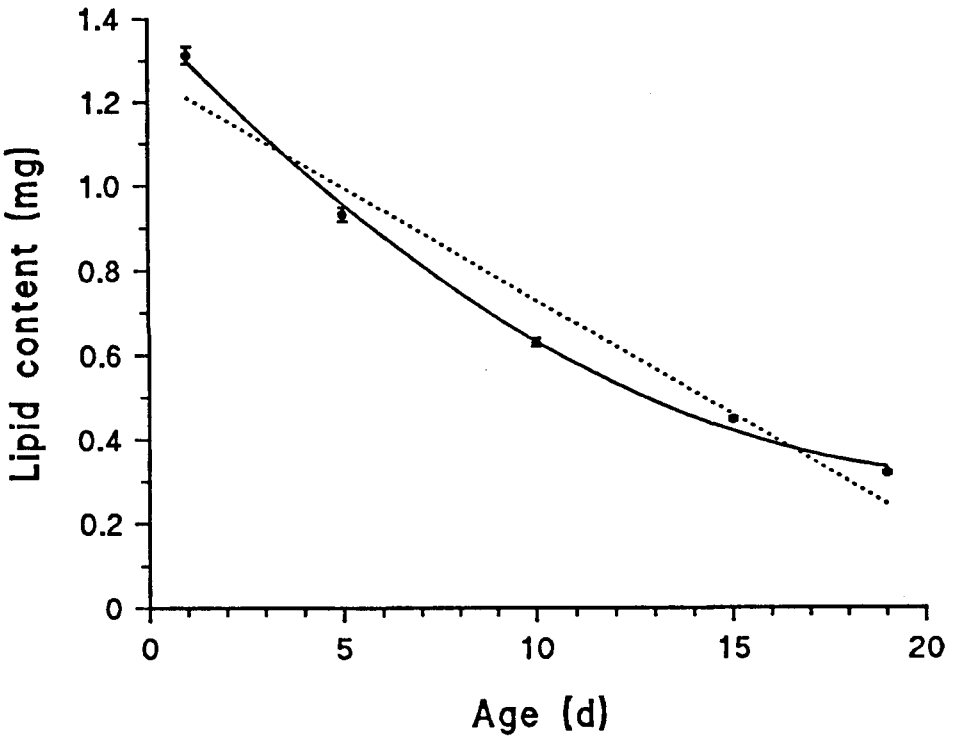

Fig. 4.6 The lipid contents of virgin females sampled at intervals after emergence (see 4.2). Points are means (+/- standard errors). Regression of means against time (d):
Lipid (mg) = 1.40 - 0.0993 t + 0.00228 t^2. $F_{2,2} = 366.6$, $p < 0.01$ (solid line).
The fitted curve was significantly non-linear: $F_{1,2} = 32.29$, $p < 0.05$. A linear regression was used for analytical purposes (see 4.4.1): Lipid (mg) = 1.26 - 0.0536 t. $F_{1,3} = 61.34$, $p < 0.01$. (dotted line).

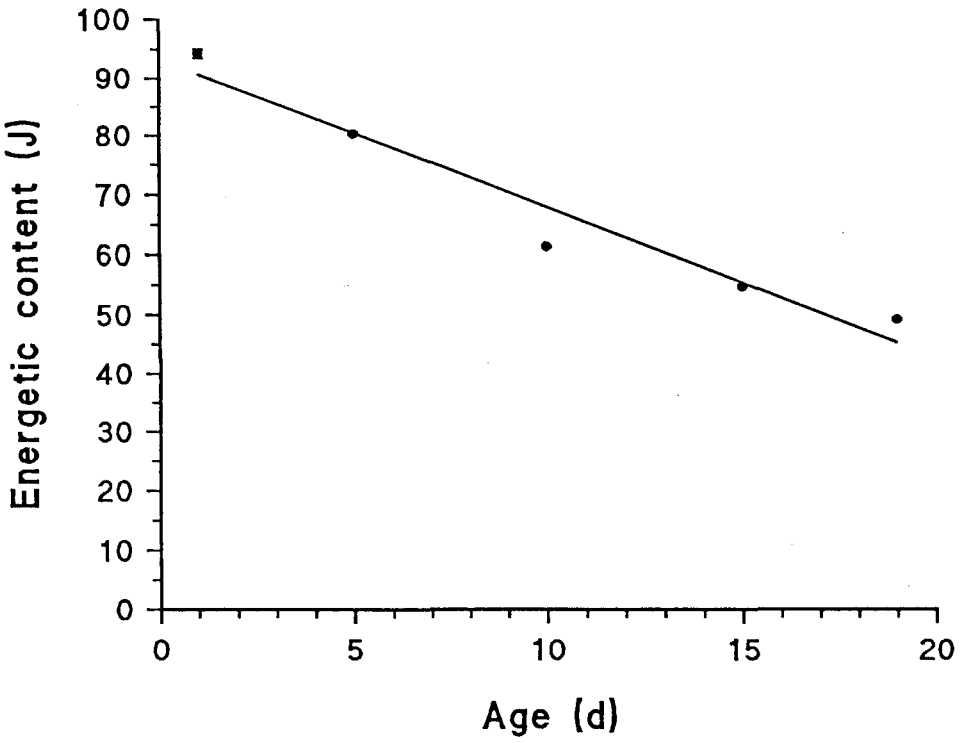

Fig. 4.7 The energetic contents of virgin females sampled at intervals after emergence (see 4.2.1; 4.2.5). Points are means (+/- standard errors). Regression of means against time after emergence (d): Energetic content (J) = 93.3 - 2.53 t. $F_{1,3} = 55.64$, $p < 0.01$.

Table 4.4 The rates of resource utilisation in virgin females. Values are abstracted from Figs. 4.4, 4.6 and 4.7 with standard deviations of the regression coefficient in parentheses.

Resource	Rate of allocation
Dry weight	84.8 (0.010) $\mu g.d^{-1}$
Potential water	97 to 137 $\mu g.d^{-1}$
Lipid	53.6 (6.85) $\mu g.d^{-1}$
Energy	2.53 (0.339) $J.d^{-1}$

emergence. Free water was not measured in cadavers but after 19 d it accounted for 58.1% of a female's fresh weight.

4.3.3. Resource content of virgin and mated females at death

There was no significant difference between the mean dry weights of the cadavers of virgin and mated females kept until death, 1.768 mg (s.e. = 0.045) and 1.858 mg (s.e. = 0.057) respectively ($t = 1.20$, d.f. = 73, $p > 0.1$). Similarly, there was no significant difference in their mean lipid contents, 0.253 mg (s.e. = 0.002) and 0.278 mg (s.e. = 0.006) respectively (Mann-Whitney U-test comparing the 7 samples of 5 females in each group: $U = 22.0$, $n_1 = n_2 = 7$, $p > 0.1$).

4.3.4. Resource allocation to reproduction

i. Estimates based on a comparison of virgin and mated females

The total amount of resource invested in producing an egg was estimated by subtracting the resource content of mated females from that of virgin females of the same age and dividing the result by the fecundity of the mated females (Table 4.5). These estimates suggested that each egg represented an investment of 4.9 µg dry weight, between 1.93 and 5.21 µg potential water, 0.9 µg lipid and 0.228 J of energy.

ii. Estimates based on the measured resource content of eggs

The mean fresh weight of eggs was 24.3 µg (s.e. = 0.038), their mean dry weight was 10.8 µg (s.e. = 0.020) and their mean free water content was 13.4 µg (s.e. = 0.025). The eggs each contained 4.72 µg (s.e. = 0.002) lipid and had a potential water content of between 19 and 24 µg. The mean energetic content of 0.312 J was estimated from measured weights of lipid and the non-lipid residuum an energy equivalence factor for lipid of 39.14 J mg^{-1} (Petrusewicz and Macfadyen, 1970, cited by Wightman, 1978) and for the non-lipid residuum of *C. analis* eggs of 20.91 J mg^{-1} (Wightman, 1978).

Table 4.5 Reproductive allocation. The amount of each resource invested in producing an egg was estimated by comparing the resource contents of virgin and mated females which had been sampled from the same population. The estimated investment in an egg was given by $(V_t - M_t)/F_t$, where, V_t and M_t are the resource contents of virgin and mated females respectively, measured at time t after emergence. F_t is the number of eggs laid by the mated female by this time. The measured resource contents of an egg (calculated in 4.3.4) are also given.

Resource	t	V_t	M_t	F_t	Estimated investment in the production of an egg	Measured resource content of an egg
Dry weight (mg)	5 d	2.33 (0.039)	1.92 (0.046)		4.91 µg	10.8 µg
Lipid (mg)	5 d	0.92 (0.001)	0.84 (0.01)	83.5 (1.97)	0.958 µg	4.72 µg
Free water (mg)	5 d	2.52 (0.049)	1.45 (0.041)			
Potential water content (mg)	5 d	3.63 - 4.84[a]	2.42 - 3.41[a]		1.9-5.2 µg[a]	19-24 µg[a]
Energy (J)	6 d	78.5 (0.169)	59.3 (0.183)	84.3 (3.46)	0.228	0.312

a: These values are the minimum and maximum estimates for potential water content which were calculated using a range of water equivalence factors (see 4.2.3.iv).

iii. The disparity between the two estimates of reproductive allocation

The measured resource contents of eggs are much greater than the estimates of resource allocation to egg production made by comparing virgin and mated females (*cf.* the last two columns of Table 4.5). The discrepancy between methods is in the same direction for all the resources measured, although the estimates differ by factors ranging between 1.4 (energy) and 4.9 (lipid). There are four possible explanations for these discrepancies: (1) measurement error in estimating the resource content of eggs or females, (2) a decline in the resource content of eggs laid by one-day old females overestimates the mean resource content of eggs, (3) creation of the resource from other resources, and (4) a difference in the rate of non-reproductive resource utilisation between laying and non-laying females. These are discussed in turn below.

1) Measurement error. As a first step in distinguishing between these explanations it is useful to compare the loss of resources from mated females over a given period (either 5 or 6 d or over the adult lifetime) with the resources put into eggs over the same period (estimated from the number of eggs laid and the measured resource content of an egg). The losses from females are equal to or greater than the resources put into eggs (Table 4.6). For all resources except lipid, the resources put into eggs represent 37 to 78% of the resources lost from females; these estimates for the resource contents of virgin, 5 or 6 day old and dead females are not inconsistent.

For lipid, the resources lost from females over 5 days (0.39 mg) are almost exactly equal to those put into eggs (0.394 mg) (Table 4.6a). This is possible if mated females allocate lipid only to eggs. In contrast, analysis over their adult lifetime suggests that the resources put into eggs represent between 37 and 44% of the lipid lost from females (Table 4.6b). These two sets of results are therefore inconsistent.

Wightman (1978) carried out a study of the resource allocation of *C. analis*, a species with a very similar life cycle to *C. maculatus*. Further analysis of Wightman's data suggests that the resources put into eggs represent 35 to 57% of the resources lost by female *C. analis* over 5 d

Table 4.6 A comparison of the loss of resources from females with the resources put into eggs over the same period. Comparisons were made either a) over a 5 d period (6 d for energy), or b) over the lifetime of the females. R_0 is the resource content of a female at emergence, M_t is the resource content of a mated female t days after emergence. V_d is the resource content of a virgin female at the time of her death.

a)

	R_0[a]	M_t[a]	Resources lost from females	Resources invested in eggs[b]	Proportion of lost resources invested in eggs
Dry weight (mg)	3.34	1.92	1.42	0.902	0.64
Lipid (mg)	1.23	0.84	0.39	0.394	1.01
Potential water (lowest estimate) (mg)	4.79	2.42	2.37	1.59	0.67
Potential water (highest estimate) (mg)	6.55	3.41	3.14	2.00	0.64
Energy (J)	92.9[h]	59.3	33.6	26.3	0.78

b)

	R_0[c]	M_d[f]	Resources lost from females	Resources invested in eggs[g]	Proportion of lost resources invested in eggs
Dry weight (mg)	3.58[d]	1.77	1.81	0.983	0.54
	3.34[e]		1.57		0.63
Lipid (mg)	1.40[d]	0.25	1.15	0.430	0.37
	1.23[e]		0.98		0.44

Source of data:

a: Mated females at emergence and after 5 d: section 4.3.4i.
b: Resource content of an egg: section 4.3.4ii; fecundity after 5 d (6 d for energy): section 4.3.4i.
c: The resource content of females at emergence was not measured in the population from which the mated females were sampled. Two estimates were available: (d) from section 4.3.2, and (e) from section 4.3.4i. Estimates of resources lost from females and the proportion of the lost resources invested in eggs were calculated using both of these estimates.
f: Mated females at death: section 4.3.3.
g: Resource content of an egg: section 4.3.4ii; lifetime fecundity: section 3.
h: This value was not measured directly but was estimated from the dry weight measurement multiplied by 27.8 $J.mg^{-1}$ the energetic content of females at emergence estimated in section 4.3.2.

(Table 4.7). The results for dry weight and energy are consistent with the results of this study. However, compared with this study, Wightman found that females invested a much lower proportion of lipid in eggs.

A comparison of the lipid results with other estimates obtained in this study suggests that the lipid content of mated females may have been substantially overestimated. The estimate of the lipid content of eggs, 43.7% dw, does not differ appreciably from Wightman's estimate of the lipid content of *C. analis* eggs, 49.8% dw (Wightman, 1978). It was estimated that, at emergence, lipid accounted for 38.7% of a female's dry weight (section 4.3.2). Thus, this sample of females would be expected to contain 1.29 mg (lipid) at emergence, a value close to the measured value, 1.23 mg. At death, lipid accounted for 15.3% of a female's dry weight (section 4.3.2). Thus, after 5 d females would be expected to contain between 0.743 and 0.293 mg lipid (between 38.7 and 15.3%). These estimates are much lower than the measured value, 0.84 mg.

Faced with this evidence it appears that the lipid content of mated females was overestimated. It is difficult to explain this. The mated females were analysed using the same procedures and the same batches of reagents as the females sampled at emergence. Moreover, the two groups of females were analysed concurrently.

2) Decline in resource content of eggs. It is possible that the resource content of eggs may have been overestimated; the sample of females from which the eggs used to measure resource content were taken may have laid larger eggs on average than the other females studied. In particular, the resource content of eggs was measured using eggs laid during the first 24 h of the females' lives; it is known that eggs decline in size with increasing maternal age (Ovenden, 1991). From the relationship between egg length and egg fresh weight, estimated by Ovenden (1991), the decline in the resource content of eggs with increasing maternal age may be estimated (Table 4.8). Assuming that the resource content of eggs is proportional to their fresh weight, the mean resource content of eggs laid by a female would be approximately 94% of the resource content of eggs laid during the female's first 24 h of oviposition. This decline is

Table 4.7 A comparison of the loss of resources from female *C. analis* over a 5 d period with the resources put into eggs over the same period. R_0 is the resource content of females at emergence, M_5 is their resource content after 5 d. These measurements of resource allocation were for females kept in containers with 50 other females, 49 males and 1000 beans which were not replaced. Data are from Wightman, 1978.

	R_0	M_5	Resources lost from females	Resources invested in eggs	Proportion of lost resources invested in eggs
Dry weight (mg)	2.93	2.09	0.84	0.48	0.57
Lipid (mg)	1.37	0.68	0.69	0.24	0.35
Energy (J)	87.23	58.08	29.15	14.28	0.49

Table 4.8 The effect of declining egg length on the estimated fresh weight of an egg. The oviposition schedule of females was estimated from 3.3.5, Experiment 4. The relationship between egg length (μm), L, and maternal age, A, was estimated by Ovenden as: L = 625.8 - 6.6A (r^2 = 0.137, n = 165), and that between egg fresh weight (μg), FW, and egg length as: FW = 0.11L - 45.58 (r^2 = 0.281, n = 29). From these equations, fresh weight was estimated as a function of maternal age. To estimate the mean resource content of eggs laid by a female it was assumed that eggs were laid at the midpoint of each interval and that their resource content was directly proportional to their fresh weight. The estimated mean resource content of eggs laid by a female during her lifetime was calculated from the mean resource content of eggs laid on each day, weighted by the number laid on that day. This estimate was 93.6% of the mean resource content of eggs laid on the first day.

Maternal age (d)	Fecundity	Fresh weight of an egg (μg)
0 - 1	26.0	22.9
1 - 2	20.9	22.2
2 - 3	16.1	21.4
3 - 4	13.8	20.7
4 - 5	7.9	20.0
5 - 6	5.1	19.3
> 6	9.4	18.5

insufficient to account for the disparity between the different estimates of the cost of egg production.

It is possible that, in addition to the decline in egg length, the resource density of eggs declines as the female ages. The importance of this effect could be determined by analysing eggs laid by females of differing ages.

3) **Creation of resources.** Insects are unable to create energy or increase their potential water content. However, they are able to synthesise lipids from non-lipid metabolites such as acetate, glycine, leucine and glucose (Clements, 1959). In some insects this may be the main source of body lipid (Gilbert, 1967). However, if non-feeding adult insects were to require a source of lipid, it would seem quite perverse for larvae to store it in a non-lipid form; this would be less compact and would impose a metabolic cost of converting the substrate on adults.

In principle, it is possible for dry weight to be created by the partial oxidation of metabolites. However, in comparison with the discrepancies observed here, any such effect would be trivial (E. Bailey, pers. comm.).

4) **Difference in the rate of non-reproductive resource utilisation.** Mated females may have had a lower rate of resource allocation to non-reproductive processes than virgin females. Plausible reasons for this include: a) virgin females may have to expend more resources searching for mates, for example, due to pheromone production (Qi and Burkholder, 1982) or greater locomotory costs; and b) there would be selection for a fertilised female with unrestricted access to oviposition sites to invest less in somatic maintenance because she will die within a week. Virgin females are likely to invest heavily in somatic maintenance to increase their longevity and hence their probability of mating.

For all resources, with the exception of lipid, the most convincing of these explanations for the discrepancies between the cost of egg production estimated using the two methods is that mated females allocate resources to non-reproductive processes at a lower rate than virgin females. For lipid, however, it seems as though the lipid content of five day old mated females has been overestimated.

4.4. Predicting the slope of the trade-off curve

Trade-off slopes may be predicted on the basis of the measurements of resource allocation for each resource in turn by assuming that the resource is limiting. These slopes are calculated by substituting measurements of the allocation of each resource into an equation which relates trade-off slope to the allocation of limiting resource. They may be compared with the slope of the trade-off curve which was measured using experimental manipulation of lifetime fecundity. If the predicted slope for a resource does not correspond with the measured slope, this resource is unlikely to be the limiting resource.

4.4.1. The relationship between resource allocation and the slope of the trade-off curve

By definition, females die when they have exhausted their available reserves of the limiting resource. A female's resource content at emergence, R_0, includes some resources which are not metabolised during her lifetime. These unavailable resources, R_d, remain in the cadaver. The reserves of a resource available to a female at emergence, R_a, are:

$$R_a = R_0 - R_d \quad (1)$$

The allocation of available reserves of the limiting resource to non-reproductive processes is the product of a female's longevity, L, and the rate at which she allocates resources to non-reproductive processes, S. The allocation to reproductive processes is the product of the cost of an egg in terms of the limiting resource, E, and the female's lifetime fecundity, F_d. The relationship between the allocation of the limiting resource, adult longevity, lifetime fecundity is therefore described by the following equation:

$$R_a = L.S + E.F_d \quad (2)$$

Rearranging Equation 2 gives:

$$L = (R_a - E.F_d)/S$$

$$= R_a/S - (E/S).F_d \quad (3)$$

which, if the rate of non-reproductive allocation is independent of a female's lifetime fecundity, is a linear equation giving adult longevity (L) as a function of lifetime fecundity (F_d), with slope, -E/S, and intercept, R_a/S. Thus, the slope of the trade-off is the cost of each egg (E) divided by the rate at which females utilise the limiting resource for processes contributing to non-reproductive processes (S). Thus, provided that S is not dependent on fecundity, the slope of the trade-off curve can be easily predicted from measured values of E and S.

In this study, an unanticipated problem was generated by the fact that the cost of egg production, estimated by comparing virgin and mated females, was substantially less than the measured resource content of eggs, suggesting that there is a difference between the non-reproductive rates of resource allocation of virgin and egg-laying females; in other words the rate of non-reproductive resource allocation is a function of fecundity ($S(F_d)$). The relationship between the reserves of the limiting resource available to a female at emergence, R_a, her lifetime fecundity, F_d, and her longevity, L, is now given by:

$$R_a = L.(S(F_d)) + E.F_d \quad (4)$$

which by rearrangement gives:

$$L = R_a/S(F_d) - \{E/S(F_d)\}.F_d \quad (5)$$

Thus, the slope of the trade-off curve between longevity and lifetime fecundity can only be predicted if the function relating the rate of non-reproductive resource allocation to lifetime fecundity is known. In this study, the rate of non-reproductive resource allocation was measured only for virgin females (section 4.3.2) and females laying about 84 eggs in 5 (or for energetic measurements 6) days; these two points are inadequate to determine the shape of this function.

In the absence of detailed knowledge of the shape of the function (Fig. 4.8), two different methods have been used to predict the slope of the trade-off. These two methods were chosen because the assumptions result in a linear trade-off between longevity and lifetime fecundity which can be predicted from the measurements made in this study.

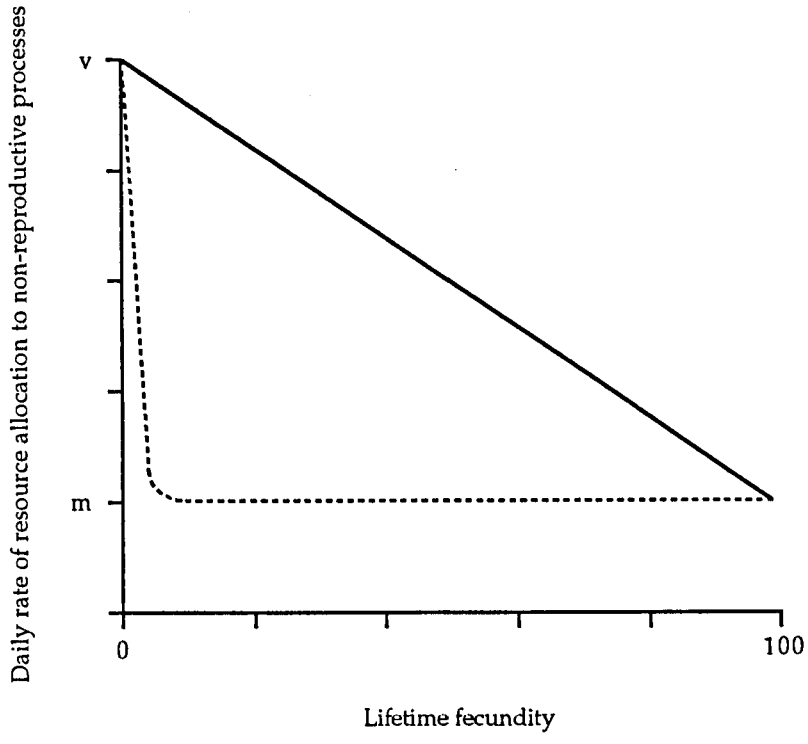

Fig. 4.8 Two types of relationship between the allocation of resources to somatic maintenance and lifetime fecundity. Laying females may allocate resources to non-reproductive processes: a) at a lower rate than non-laying females, unaffected by their lifetime fecundity (dashed line) or b) at a rate dependent on their lifetime fecundity (solid line). Virgin females allocate resources to somatic maintenance at a rate **v**, the lowest rate of allocation of resources to somatic maintenance by mated females laying up to 100 eggs is **m**.

i. Assuming that lifetime non-reproductive resource allocation is reduced by a constant amount for each egg laid

If each egg laid reduced the lifetime non-reproductive allocation (rather than the daily non-reproductive allocation) by a constant amount, R_e:

$$R_a = (L.S_v - R_e.F_d) + E.F_d \quad (6)$$

where S_v is the rate of non-reproductive resource allocation in virgins. This equation may be rearranged to give a linear relationship between adult longevity and lifetime fecundity:

$$L = R_a/S_v - \{(E - R_e)/S_v\}.F_d \quad (7)$$

Thus the slope of the trade-off curve is $(E - R_e)/S_v$. There is no obvious biological mechanism which corresponds with the assumption that each egg reduces the total non-reproductive allocation (rather than the rate of non-reproductive allocation) by a constant amount, but the assumption does at least provide a means by which the slope of the trade-off may be predicted.

The net cost of each egg, $E - R_e$, may be calculated by comparing the resource contents of mated and virgin females after a given time:

$$(V_t - M_t)/F_t = \{(R_0 - S_v.t) - (R_0 - [S_v.t - R_e.F_t] - E.F_t)\}/F_t$$

$$= (E - R_e) \quad (8)$$

Thus the estimate of investment per egg obtained by comparing the resource contents of virgin and mated females (Table 4.9a) is equivalent to $(E - R_e)$. S_v was estimated as the slope of the linear regressions of the resource content of virgins on age (Table 4.4).

ii. Assuming that the rate of non-reproductive resource allocation (in females laying more than a few eggs) and the daily cost of egg production are independent of lifetime fecundity

If the non-reproductive rate of resource allocation declines rapidly with increasing lifetime fecundity initially, but is independent of lifetime fecundity in females laying more than a few

Table 4.9 Calculation of the predicted trade-off slopes. Predictions were made on the assumption that a) lifetime non-reproductive resource allocation is reduced by a constant amount for each egg laid, or b) the rate of non-reproductive resource allocation and the daily cost of egg production are independent of lifetime fecundity. For explanations of the symbols used and of how each quantity was calculated see section 4.4. The measured trade-off slope was - 0.105 d.egg^{-1} (s.e. = 0.021).

a)	Resource	Net cost of each egg	Non-reproductive rate of resource allocation	Predicted trade-off slope
		$E - R_e$	S_v (d^{-1})	$-(E - R_e)/S$ ($d.egg^{-1}$)
	Dry weight (μg)	4.91	84.8	- 0.058
	Lipid (μg)	0.958	53.6	- 0.018
	Potential water (μg)	1.9 to 5.2	97 to 137	- 0.122 to - 0.146
	Energy (J)	0.228	2.53	- 0.090

b)	Resource	Resource content of each egg,	Net metabolic rate of egg-laying females	Predicted trade-off slope
		E_c	$S_l + C_e$ (d^{-1})	$-E_c/(S_l + C_e)$ ($d.egg^{-1}$)
	Dry weight (μg)	10.8	104	- 0.104
	Lipid (μg)	4.72	-0.0424	+ 5.73
	Potential water (μg)	19 to 24	156 to 227	- 0.121 to - 0.106
	Energy (J)	0.312	1.21	- 0.207

eggs (Fig. 4.8, dashed line), and the daily cost of egg production (over and above their resource content) was independent of the number of eggs laid, then:

$$R_a = L.S_l + (L.C_e + E_c.F_d) \quad (9)$$

where S_l is the rate of non-reproductive resource allocation of laying females, C_e is the daily cost of egg production and E_c is the resource content of an egg. By rearrangement:

$$L = R_a/(S_l + C_e) - \{E_c/(S_l + C_e)\}.F_d \quad (10)$$

Thus the slope of the trade-off is $E_c/(S_l + C_e)$. The resource content of eggs, E_c, has been measured (see Table 4.5), and $(S_l + C_e)$ can be estimated from the resource contents of newly emerged females, R_0, and mated females at time t, M_t:

$$R_0 - M_t = R_0 - \{R_0 - (S_l + C_e).t - E_c.F_t\} \quad (11)$$

By rearrangement:

$$(S_l + C_e) = (R_0 - M_t - E_c.F_t)/t \quad (12)$$

R_0 and M_t were measured in females randomly sampled from the same population (Table 4.5). M_t was measured for t = 5 (t = 6 for energy). Values of $(S_l + C_e)$ and E_c are given in Table 4.9b.

4.4.2. Calculation of predicted trade-off slopes for each resource

Trade-off slopes could only be predicted if the resource content of mated females, M_t was measured a given time after emergence. Given the innaccuracy of either the measurement of the mated female's lipid content 5 d after emergence or the measured lipid content of eggs (see 4.3.4iii), the trade-off slopes predicted on the assumption that lipid was the limiting resource are not discussed (however, these values are given in Table 4.9).

Predictions for the trade-off made for each resource differed in magnitude as much within each assumption as between assumptions. When the trade-off slope was predicted assuming that each egg reduced the total non-reproductive resource allocation by a constant amount (Assumption i, Table 4.9a), the predicted values differed in magnitude by a factor of

approximately 2.5. These predicted trade-off slopes ranged from being shallower (dry weight, energy) to being steeper (entire range of estimates for potential water) than the measured trade-off slope. Similarly, when the trade-off slope was predicted assuming that both the reduction in the rate of non-reproductive resource allocation in females laying more than a few eggs and the daily cost of egg production were independent of the female's lifetime fecundity (Assumption ii, Table 4.9b), the predicted values again differed by a factor of approximately 2.5. A between assumption comparison of the trade-off slopes predicted for each resource reveals similar differences: predicted trade-off slopes differ in magnitude between assumptions by a factor of between 1.2 (minimum estimate for potential water) to 2.8 (energy). It seems therefore that assumptions about the relationship between the non-reproductive rate of resource allocation and lifetime fecundity have too important an influence on the predicted slope of the trade-off curve for any useful conclusions to be drawn from these predicted slopes without an understanding of this relationship.

4.5. Discussion

4.5.1. Resource allocation in *C. maculatus*

The resource composition of female *C. maculatus* at emergence is 3.46 mg free water, 1.40 mg lipid and 2.18 mg non-lipid dry weight. They have a mean energetic content of 99.6 J. By their death, virgin females have utilised 81% of this lipid and 60% of the non-lipid dry weight and 57.5% of their energetic content. Females have a mean lifetime fecundity of 91 eggs (see 3.3.1, Experiment 1). As a proportion of their resource content at emergence, this reproductive allocation represents 30.1% of the lipid (if the measured lipid content of an egg was accurate), 25.4% of the non-lipid dry weight and 28.5% of the energy. Analysis of water budgets is less reliable both because water content at the time of death cannot be measured and because the amount of water produced by the metabolism of other resources is not known. However, assuming that metabolism of 1 mg lipid releases 1.07 mg water (Brody, 1945), at emergence females have a potential water content of between 5.4 and 6.7 mg water depending on the value of the water equivalence factor chosen for non-lipid dry weight (N = 0.2 to 0.8). By

death, the potential water content of virgin females has declined by approximately 40%. If females laid 91 eggs this would represent a loss of approximately 32% of their potential water content.

The results of previous studies of the resource contents of females in other populations of *C. maculatus* are broadly in agreement with the results of this study (Table 4.10). Only two results differ appreciably from those of this study. The first of these is the lower energetic content, 22.2 J/mg dry weight, measured by Chandrakantha and Mathavan (1986). This value was obtained for females which had been oven dried at 90°C, which would have evaporated many of the volatile lipids. The second is the lower lipid content measured by Sharma *et al.* (1983). This value was obtained for mated females. However, the eggs produced during the first day could not account for this lipid discrepancy. It is possible that this is a real between population difference. However, Downer (1985) has argued that, in the absence of a standardised extraction procedure it is unwise to infer too much from between study comparisons.

The proportions of dry weight to fresh weight and of lipid content to dry weight are very similar in *C. maculatus* and *C. analis* eggs (Table 4.11). *C. analis* adults are smaller than *C. maculatus* females (Table 4.10); therefore, the larger size of *C. analis* eggs is not a consequence of differing adult body size. One could speculate that the strategy of larval *C. analis* to attack larvae cohabiting within a bean increases the benefits associated with larger larvae and hence larger eggs, more than the strategy of larval *C. maculatus* to avoid cohabiting larvae (see Smith and Lessells, 1985). This hypothesis is supported by the finding that the eggs of 'attack strategy' *C. maculatus* are larger than 'avoid strategy' *C. maculatus* (Messina, 1991). An alternative explanation is that Wightman's *C. analis* stock culture was adapted to being kept on dwarf green peas at 29°C and 25% rh rather than on black-eyed beans at 30°C and 70% rh; the optimal larval size may be greater under these conditions.

Comparing this study with Wightman's (1978) data, it would appear that *C. maculatus* females invest a greater proportion of their available resources in eggs during their lifetimes than *C. analis* females (Table 4.12). However, *C. maculatus* females were studied in the absence of other females and with a daily supply of fresh beans. *C. analis* were studied in containers

Table 4.10 Resource content of female *Callosobruchus* species at emergence or shortly thereafter. A comparison between this study and previous studies of the resource content of adult female *Callosobruchus* spp. Age is measured from emergence. Values are mean resource contents of females. Water content is expressed as a percentage of fresh weight, fw. Lipid content is expressed as a percentage of dry weight, dw. Energetic content is expressed as J / mg dry weight. Females were mated soon after emergence in all studies except this one.

Species	Age (d)	Dry weight (mg)	Water content (% fw)	Lipid content (% dw)	Energetic content (J $mgdw^{-1}$)	Source
C. *maculatus*	1	3.46	49	38.0	27.0	This study
C. *maculatus*	1		51	37.0		a
C. *maculatus*	1	3.27	52			b
C. *maculatus*	0	2.9			22.2	c
C. *maculatus*	1			30.3		d
C. *analis*	0	2.91	48	47.1	30.0	e

a: Nwanze *et al.*, 1976
b: Sharma and Sharma, 1984
c: Chandrakantha and Mathavan, 1986
d: Sharma *et al.*, 1983
e: Wightman, 1978

Table 4.11 Resource content of *Callosobruchus* eggs. A comparison between this study and previous studies of the resource content of *Callosobruchus* eggs. Values are means with standard errors.

Species	Fresh weight (µg)	Dry weight (µg)	Lipid content (% dw)	Energetic content (J $mgdw^{-1}$)	Source
C. *maculatus*	24.28 (0.038)	10.84 (0.020)	43.7	0.0288[a]	This study
C. *analis*	36.7[b]	17.6	49.8[c]	0.0298[d]	Wightman, 1978

a: Estimated using the method described in 4.3.4

b: Calculated from the values for egg dry weight (17.6 mg) and dry weight: fresh weight ratio (0.48)

c: Estimated by Wightman (1978)

d: Calculated from the values for egg dry weight and energetic equivalent (29.76 J mg^{-1})

Table 4.12 Lifetime resource investment in eggs. A comparison between *Callosobruchus maculatus* (data from Tables 4.4 - 4.5) and *C. analis* (data from Wightman, 1978) of their lifetime resource investment in eggs. Values are expressed as the proportion of the resources available to the female at emergence (R_a) which is incorporated into eggs during her lifetime, $(E_c \times F) / R_a$.

	Lifetime fecundity	Dry weight investment	Lipid investment	Energetic investment
C. maculatus	91.0[a]	0.56	0.38	0.56
C. analis	34.0[b]	0.38	0.24	0.34
C. analis	60[c]	0.69[d]	0.45[d]	0.59[d]

a: The mean fecundity of females ovipositing alone with daily bean replacement

b: The mean fecundity of 51 females kept with 49 males ovipositing on 1000 beans which were not replaced; the conditions under which Wightman (1978) made all the measurements of resource allocation cited in this study.

c: The mean fecundity of females ovipositing alone with daily bean replacement (see text)

d: Calculated using Wightman's (1978) estimates of resource allocation

with 51 females and 49 males on 1000 beans which were not replaced. In the absence of other adults, with a daily supply of fresh beans, *C. analis* females have a mean lifetime fecundity of approximately 60 eggs (N. Colegrave pers. comm.). This estimate of lifetime fecundity, combined with Wightman's resource allocation data, gives estimates of lifetime resource investment for *C. analis* which are slightly greater than the equivalent estimates for *C. maculatus* (Table 4.12).

4.5.2. Predicting the slope of a trade-off from measurements of resource allocation

Determining the limiting resource which caused the trade-off between adult longevity and lifetime fecundity from measurements of resource allocation was an exciting prospect. It had seemed that *C. maculatus* was an ideal species in which this aim might be achieved. While there were inevitable problems which would limit the power of this approach to distinguish between resources under certain conditions, the unexpected finding that the rate of resource allocation to non-reproductive processes was substantially lower in mated than in virgin females undermined it.

Without an understanding of the relationship between the rate of resource allocation to non-reproductive processes and a female's lifetime fecundity, predicted trade-off curves cannot be conclusive. At first sight it would seem that this relationship could be determined by experimentally manipulating lifetime fecundity in otherwise similar groups of females and comparing their resource contents after a given time. However, the rate of resource allocation to non-reproductive processes would not be the only variable influenced by such experimental manipulations. The total cost of an egg includes both its resource content which can be measured and an extra metabolic cost which must be estimated. It is likely that the metabolic cost of each egg is also a function of lifetime fecundity, $E(F_d)$. The resource content of a mated female at time, t, after emergence would then be given by:

$$M_t = R_0 - S(F_d) - \{E(F_d) + E_c\}.F_t \qquad (13)$$

Thus, the extra metabolic cost of an egg cannot be measured independently of the reduction in non-reproductive resource allocation. Consequently, assumptions about the extra metabolic

cost of each egg are required before the relationship between the non-reproductive rate of resource allocation and lifetime fecundity may be estimated. It is not clear that this is a step forward. Therefore, in *Callosobruchus*, at least, there is no way of measuring the relationship between non-reproductive resource allocation and lifetime fecundity independently of the extra metabolic cost of egg production.

The second problem which reduces the power of this approach is the assumption, implicit throughout this study, that all processes share a common pool of resources. This may not be so; for example, some compounds, such as membrane proteins, may be used for egg production but be unavailable for somatic maintenance. This problem might be solved by radioactively labelling compounds in the adult and monitoring their uptake. This would determine whether any compounds were selectively used for one process only. Whilst radioactive labelling would be difficult in this species because the adults do not feed, it ought to be possible to label the host bean and hence the larvae.

Third, more than one of the predicted trade-off slopes may be similar to the measured trade-off slope. For example, when it was assumed that the rate of non-reproductive resource allocation was unaffected by lifetime fecundity (Assumption ii), predicted trade-off slopes based on either water or dry weight were similar to the measured slope. This similarity suggests that these resources are allocated in the same ratio to reproductive and non-reproductive processes and that one or both of them are limiting. If only one were limiting, there seems to be no way of determining which.

Fourth, the resources measured may be inter-related. In this study, for example, the dominant component of dry weight is lipid which has a higher metabolic yield per unit mass of both water and energy than most other components of dry weight. Arguably, given the errors involved, measurements of energy and water allocation are simply alternative ways of measuring lipid allocation. This argument can be pursued to suggest that measurement of any two of these resources are simply alternative methods of measuring allocation of the third, albeit slightly inaccurate methods. It would not be surprising therefore if the predicted trade-off slopes were similar. One way of avoiding this problem would be to measure the allocation of

elements rather than compounds. However, this solution would obviously be hopeless if the limiting resource were in fact a compound.

Fifth, the rate of resource utilisation in virgins was estimated using a linear regression of resource content on age after emergence. However, a quadratic regression explained the relationship between female age and lipid content significantly better than a linear regression; using a linear regression reduced the accuracy of the estimate. Either a female's age or her declining resource content could be responsible for any changes in her rate of resource utilisation; these data were inadequate to distinguish between these causes. Consequently, any attempt to incorporate changing rates of resource utilisation would have introduced new sources of error.

If this approach is flawed for this species, are there are other approaches to determining the limiting resource? If the trade-off were caused by a single limiting resource, the trade-off should be unaffected by environments which differed in other resources. The adult longevity and lifetime fecundity of female *C. maculatus* increase when they are fed with a range of different nutrients, from sugar solutions to yeast extract solutions (Møller *et al.*, 1989b). This suggests that the trade-off is caused by limitation in a resource common to these solutions such as energy or water. It is difficult to distinguish between these resources using this approach. All energetically important nutrients would be metabolised to release water and feeding adults water would have energetic consequences.

An alternative method of determining the limiting resource is to experimentally manipulate the lifetime fecundities of groups of females and then to analyse the resource contents of the cadavers. Groups of cadavers would be expected to differ in their contents of all resources except the limiting resource. When the resource contents at death of mated and virgin female were compared in this study, there were no significant differences between their mean dry weights or their mean lipid contents and hence between their potential water contents. These results again suggest either that females allocate resources in the same ratio to non-reproductive and reproductive processes or that resources are interchangeable and that females arrange their allocation to exhaust all resources simultaneously.

As a technique of determining the limiting resource, comparing the resource content of cadavers is still limited by most of the problems which were outlined above. If processes do not share a common pool of resources, females with differing lifetime fecundities may die with differing sized reserves of the limiting resource which are inaccessible to non-reproductive processes. If resources are allocated in the same ratio to reproductive and non-reproductive processes, this technique will not distinguish between them. If lipid, energy and dry weight measurements were more or less equivalent, one would not expect them to differ at death.

Nevertheless, the best approach to determining the limiting resource appears to be to compare the resource contents of cadavers which are experimentally manipulated so that they lay different numbers of eggs. It would have been instructive to be able to relate the trade-off between adult longevity and lifetime fecundity to patterns of resource allocation. For example, if two populations were known to differ in the mean resource contents of eggs, predictions could be made about whether or not the shape of the trade-off should differ between them. Without a knowledge of the relationship between the trade-off and patterns of resource allocation, such predictions are impossible. However, determining this relationship seems impossible, at least in *C. maculatus*. Two major problems were encountered in this study: the apparent reduction in the rate of resource allocation to non-reproductive processes in mated females and, related to this, the problem of separating the metabolic cost of producing eggs from this non-reproductive allocation. These problems prevent the cost of reproduction being estimated by measuring resource allocation in this species. Importantly, they cast doubt on estimates of the cost of reproduction made for other species where non-reproductive allocation was measured in non-breeding females.

5. The cost of reproduction for males

5.1. Introduction

5.1.1. Are males likely to suffer a cost of reproduction?

A female's reproductive success is directly affected by the amount she invests in her offspring; she can determine both their number and, according to her investment in each of them, their fitness (Ovenden, 1991). In *Callosobruchus maculatus*, for example, a typical clutch of 90 eggs represents an investment of approximately 30% of the female's emergence body weight (Chapter 4).

The mass of sperm used to fertilise this clutch of 90 eggs is trivial in comparison with the female investment. Historically, it has been argued that, given this lack of reproductive investment, males suffer negligible costs of reproduction (Bateman, 1948). However, it has since become clear that males produce non-trivial amounts of ejaculate (Dewsbury, 1982). These ejaculates typically include far more sperm than the amount used to fertilise eggs (a single *Callosobruchus maculatus* spermatophore may contain up to 80,000 sperm; Eady, 1992). They also contain large quantities of accessory substances which may have roles in either ensuring fertilisation or increasing female fecundity (for reviews see Parker, 1970; Leopold, 1976; Chen, 1984; Parker and Simmons, 1989). Furthermore, reproduction may involve significant non-copulatory investment by males, such as searching for females, competing with other males and courtship. Sustaining copulation, which can last up to 3 minutes in this species (pers. obs.), may also be energetically costly.

5.1.2. Causes of the costs of reproduction

The cost of reproduction to male invertebrates has most frequently been studied by restricting their exposure to females and hence controlling the number of times that they copulate. These experimental manipulations have often shown that males pay a cost of reproduction in terms of reduced longevity (*Drosophila melanogaster*: Bilewicz 1953, Malick and Kidwell 1966,

Partridge and Farquhar 1981, Partridge and Andrews 1985, Service 1989; true armyworm *Pseudaletia unipunctata*: Fitzpatrick and McNeil 1989; the nematodes *Caenorhabditis elegans*: van Voorhies 1992 and *Panagrellus redivivus* under some conditions: Abdulrahman and Samoiloff 1975). However, other studies have shown no effect of a male's mating history on his expected longevity (*Drosophila melanogaster*: Kidwell and Malick, 1967; brine shrimp *Artemia*: Browne 1982; monarch butterfly *Danaus plexippus*: Oberhauser 1989; speckled wood butterfly *Pararge aegeria*: Svärd 1985). Moreover, Kidwell and Malick (1967) found that, under certain conditions, mated male *Drosophila melanogaster* lived longer than virgins.

There are two problems with this approach. First, whereas manipulating the availability of oviposition sites resulted in an easily quantifiable change in the reproductive effort of female *Callosobruchus maculatus*, the relationship between male reproductive effort and the availability of females is much harder to quantify. For instance, the number of copulations achieved by a male would not reflect their cost if ejaculate size varied. Second, it is difficult to distinguish between the different interactions of males and females; manipulating the access of males to females simultaneously affects their non-reproductive competitive interactions, their opportunity for courtship and their opportunity for copulation (Partridge and Farquhar, 1981).

These components of male costs of reproduction have been studied in *Drosophila melanogaster*. The longevity of virgin male *Drosophila melanogaster* is reduced by exposure to females (Partridge and Farquhar, 1981). The survival probability of a male depends on whether or not he is currently with females, not on his past exposure to females (Partridge and Andrews, 1985). This suggests that reproduction reduces male longevity because of the physiological risk rather than accelerated senescence. Furthermore, the fertility of males is unaffected by their previous mating experience (Partridge, 1987).

To separate the effects of courtship from copulation, females which are courted but which will not copulate are needed. In *Drosophila melanogaster* there is no evidence that males courting unreceptive females have a shorter longevity than virgin males kept alone (Partridge and Farquhar, 1981). However, these courtship costs may be minimal compared with males courting receptive females because in *Drosophila melanogaster*, unreceptive females

terminate courtship by extruding their ovipositor. When females are removed from males kept with females since eclosion, there is a lag before the life expectancy returns to that of males kept without females since eclosion (Partridge and Andrews, 1985). Partridge and Andrews (1985) speculated that this might be a consequence of the time taken to replenish accessory glands or testes, and hence that the resource allocation to the ejaculate caused the cost of reproduction in males. This would explain why, when females are provided for previously celibate males, no such lag is observed before their life expectancy falls to that of males kept with females since eclosion; resource diversion would commence as soon as females were introduced.

5.1.3. Payment of the costs of reproduction

Reduced male longevity is not neccessarily evidence of a cost of reproduction. The cost of reproduction is, by definition, a reduction in future reproduction as a consequence of current reproduction. Longevity is only relevant if increased longevity reflected increased opportunity for future reproduction; one would have to demonstrate that the males which had been given limited exposure to females were still reproductively active after the other males had died. Conversely, if reproductively active males do not have a reduced longevity, this is not evidence that they do not suffer a cost of reproduction; they may be less able to secure copulations or fertilise the female after reproduction even if their longevity were unaffected.

The ways in which the cost of reproduction for male insects is paid can be divided into five categories. Current reproduction may reduce a male insect's subsequent ability to:

1) Secure copulations. This could result from the male's inability to compete successfully with other males, to court the female adequately or to penetrate a receptive female successfully. It is difficult to distinguish between the possible reasons for a male not remating; they may court females inadequately either because they are unable to court them well or because they have little to gain from copulation. There are cases which suggest that males are not remating because they cannot court the female. For

example, Simmons (1990) found that the refractory period before remating in male zaprochilines (Tettigoniidae) was correlated with replenishment of their spermatophylax. This may be because these males would not be successful in courting the female; it is known that female Tettigoniids choose males which provide the largest spermatophylax (*e.g.* Gwynne, 1982).

2) Produce a fertile ejaculate. This could be due either to a lack of sperm inseminated with the ejaculate (Outram, 1971; Fitzpatrick and McNeil, 1989) or perhaps to a deterioration in sperm quality caused by a lack of protective ejaculate components. For example, Outram (1971) showed that spermatophore fetility in *Choristoneura fumiferana* (Lepidoptera: Tortricidae) declined with successive copulations with virgin females.

3) Produce a competitive ejaculate. A male will benefit if he can increase the likelihood that his ejaculate rather than any other is used to fertilise the female's eggs. The mechanisms of inter-ejaculate competition are poorly understood but both ejaculate size and composition are likely to be be important; many studies have shown that mating reduces the size of subsequent ejaculates (Rutowski *et al.*, 1987; Svärd, 1985; Svärd and Wiklund, 1986,1989; Oberhauser, 1988). For example, the ejaculates of recently mated *Colias eurytheme* (Lepidoptera: Pieridae) contained only about 40% of the material contained in the ejaculates of previously virgin males (Rutowski *et al.*, 1987).

4) Produce an ejaculate which effectively manipulates female reproductive behaviour or physiology. The male may use the ejaculate to manipulate female reproduction in one of two ways. He may increase either: a) the speed with which the female matures eggs and oviposits; or b) the period before she remates. These manipulations both reduce the amount of lost paternity should the female subsequently remate and fertilise eggs with sperm from another male. They may be mediated by either ejaculate composition or morphology (for reviews see Leopold, 1976; Chen, 1984). The ejaculates of previously mated males are less effective in manipulating female reproduction in many insects (Leopold et al., 1971; Oberhauser, 1989; Markow et al., 1990; Svärd and Wiklund, 1991; *C. maculatus:* Eady, 1992). For example, by restricting the flow of haemolymph to the brain at varying times with respect to mating, Leopold *et al.* (1971)

showed that male accessory gland secretions were responsible for inducing mating refusal following copulation in female *Musca domestica*. They then showed that mating refusal in females was less frequent if the males had previously mated; previous mating had made these ejaculates less effective at manipulating female reproductive behaviour.

5) Produce an ejaculate which increases female fecundity. An increase in female fecundity would benefit the male whose sperm fertilise the eggs (For a discussion of the mechanisms by which this may be achieved see Chapter 6). Studies have shown that female lepidopterans mating with previously mated males have a lower fecundity. In several cases it has been shown that this is unlikely to be due to sperm depletion and is probably due to the declining nutrient content of the ejaculate (*e.g. Danaus plexippus, Heliconius hecale* and *Heliconius erato* (Lepidoptera: Nymphalidae): Boggs and Gilbert, 1979; *Colias eurytheme* (Lepidoptera: Pieridae): Rutowski et al., 1987).

5.1.4. Aims

The aims of this chapter were:

1. To investigate a possible cost of reproduction to males.

2. To distinguish between the causes of the cost of reproduction for males: copulation, courtship or competitive interactions.

3. To investigate the ways in which males pay this cost of reproduction.

5.2. Methods

5.2.1. Distinguishing between the causes of the cost of reproduction: experimental manipulation

i. Copulatory or non-copulatory causes

Costs of reproduction could be incurred due to copulation or courtship. These possible causes were separated by manipulating male reproductive behaviour by confining males alone or with females which differed in their receptivity. Virgin males, between 15 and 24 h after emergence, were randomly allocated to one of the following 3 groups which differed in the mating status of female(s) placed with the male:

Group 1: no accompanying beetles.

Group 2, 'courting': 1 just-mated female for 1 h.

Group 3, 'courting and copulating': 1 just-mated female for 45 min followed by 1 virgin female for 15 min.

'Just-mated' females are females which had copulated less than 15 min before being placed with an experimental male; these females were courted by males but refused to mate with the experimental male (R. Holt, in prep.). Thus, comparisons between groups 1 and 2 reflect the non-copulatory costs of being with females; the costs of competition with them and of courting them. Comparisons between groups 2 and 3 reflect the costs of copulation; these groups differed only in copulation opportunities, they courted females for similar amounts of time (but see discussion).

Approximately 220 males were allocated to group 1, and 17 to each of groups 2 and 3. The sample size of 17 reflected the maximum number of pairs of beetles which could be reliably monitored simultaneously. Males were kept individually in upturned 5 cm Petri dishes into which females were introduced 3 times each day as described above; at 1000, 1200 and 1500 h. All copulations were recorded and the first virgin females placed with group 3 males each day were subsequently placed with 20 beans in 25 ml containers. Their lifetime fecundity was scored; the number of infertile eggs and number of larvae dying during the first

instar were also recorded. A predetermined and randomly selected sample of 20 males from group 1 and all males from groups 2 and 3 were checked at 24 h intervals to determine their longevity. After their death the elytral lengths of both males and ovipositing females were measured.

Changes in the fertility of the group 1 males were monitored: at 1000 h each day, a random sample of 15 group 1 males were placed individually under upturned 5 cm Petri dishes, each with a virgin female from the same stock culture and age as those placed with the group 3 males above. If copulation took place the female was placed on 20 beans and her lifetime fecundity was scored as above. There were insufficient males in group 1 to continue sampling males at this rate throughout their lives; after the tenth day, ten males only were sampled at 48 h intervals.

ii. Courtship or competitive causes

The two non-copulatory costs of being with females, courtship and competition, were distinguished by placing virgin black morph males, 1 - 15 h from emergence, in 25 ml containers alone, with virgin normal males or with just-mated females:

Group 1: no accompanying beetles

Group 2 'competing': 1 accompanying virgin male for 1 h

Group 3 'courting': 1 accompanying just-mated female for 1 h

Fifteen males were allocated to each group. They were kept individually in upturned 5 cm Petri dishes into which accompanying beetles were introduced 3 times each day, at 1000, 1200 and 1500 h. Males were checked at 12 h intervals to determine their longevity. Black morph experimental males were used so that they could be distinguished from accompanying males.

5.2.2. The effect of copulation on the size of subsequent ejaculates

Fifteen virgin males, 15-24 h after emergence, were individually placed under Petri dishes. Virgin females of the same age were anaesthetised using carbon dioxide, weighed to the nearest μg and placed either with one of the males or under Petri dishes alone. All copulations

taking place over the next 30 min were recorded. After this 30 min period the females were again anaesthetised and weighed. Metabolic weight loss was calculated from the change in weight of the virgin females. Ejaculate weight was calculated from the change in mass of the mated females after correcting for metabolic weight loss.

The procedure was repeated twice more using fresh virgin females but the same males to investigate the effect of previous copulations on ejaculate mass. Only males which had copulated on all previous exposures to females were included in the analysis.

5.2.3. The effects of repeated copulation

Twenty virgin males, between 36 and 48 h after emergence, were individually placed under upturned 5 cm Petri dishes with a virgin female, also between 36 and 48 h after emergence. Following copulation the female was removed and immediately replaced with another virgin female. Females were replaced in this way for four hours. Following her removal, each female was put in a 25 ml container with 5 beans which were replaced four times at 24 h intervals with 5 fresh beans.

The fecundities of the females on each of their first 4 d of oviposition and thereafter were scored. The number of infertile eggs and the number of larvae failing to develop past the first instar were recorded separately. The elytral lengths of males were measured.

In a similar experiment, to assess any long term effects of repeated mating, 40 virgin males, aged between 36-48 h after emergence, were randomly allocated between two groups. Males in the first group were treated as before on the first day. 20 h later they were mated to a maximum of 4 females. Males in the second group were not mated on the first day but were repeatedly mated on the second day to a maximum of 6 females. Females were placed on 20 beans following a successful copulation. These beans were not replaced and lifetime fecundity was recorded.

5.3. Results

5.3.1. Distinguishing between the causes of the cost of reproduction: experimental manipulation

i. Copulatory and non-copulatory causes

Males allowed to court for three 1 h bouts per day but unable to copulate died sooner than virgin non-courting males. Males allowed to court and copulate died sooner than both other groups of males (Fig. 5.1). There was no significant difference in the sizes of males, as measured by elytral length, between the three groups (Kruskal-Wallis 1-way Anova: Chi-square = 0.4604, d.f. = 2, $p > 0.5$).

For 7 d the fertility of the thrice-daily mated males (group 3) remained indistinguishable from that of the hitherto virgin males of the same age (group 1). Thereafter, the fertility of the group 3 males declined until after 11 d they were incapable of further fertile copulations. This decline is apparent even when analysis excludes infertile copulations, but the real cost of reproduction, including infertile copulations and missed opportunities for copulation, is higher (Fig. 5.2).

ii. Courtship and competitive causes

Males which were kept alone lived significantly longer than either males allowed to court females but unable to copulate, or males placed with other males (Kruskal-Wallis 1-way Anova: Chi-square = 21.9, d.f. = 2, $p < 0.001$). There was no difference in the longevity of males allowed to court females but unable to copulate and those placed with other males (Fig. 5.3)

5.3.2. The effect of copulation on the size of subsequent ejaculates

Ejaculate mass declined in successive copulations (Table 5.1). The first copulation resulted in a mean ejaculate mass of 227 µg, approximately 4% of male emergence weight. By the third copulation ejaculate mass had declined to 74 µg.

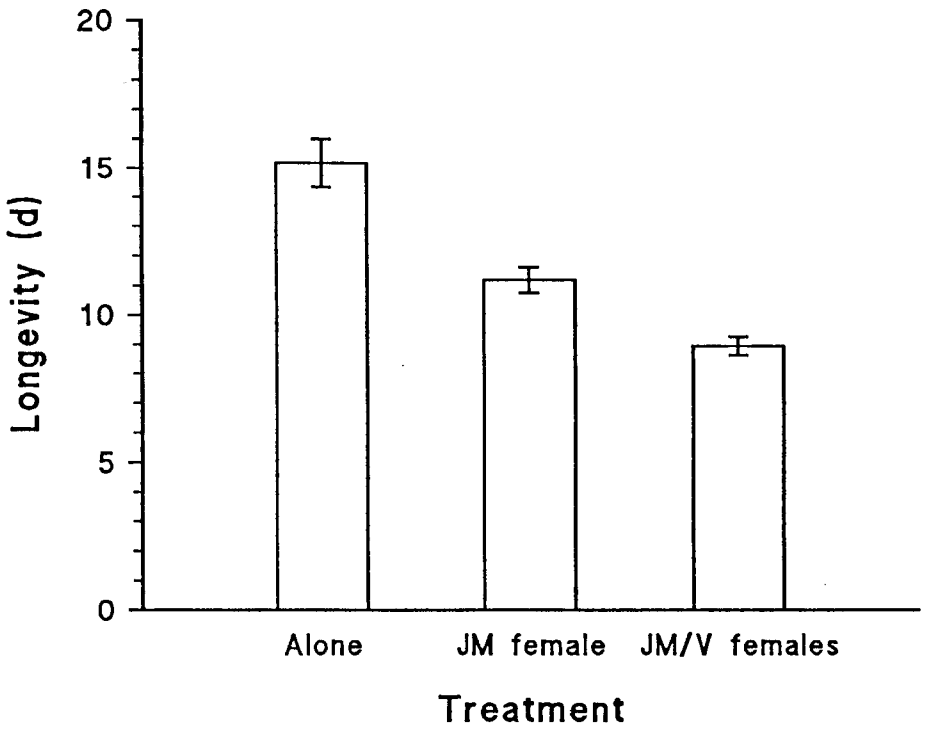

Fig. 5.1 The longevities of males kept alone (Group 1), able to court (Group 2) or able both to court and copulate (Group 3). Treatment mean longevities differed significantly (ANOVA: $F_{2, 50} = 26.07$, $p < 0.001$). The mean longevities of males in Group 1 differed significantly from those of both Groups 2 and 3 (GT2 unplanned multiple comparisons among pairs of means based on unequal sample sizes: Group 1 *vs.* Group 2, $p < 0.01$; Group 1 *vs.* Group 3, $p < 0.01$, Group 2 *vs.* Group 3, $p < 0.05$).

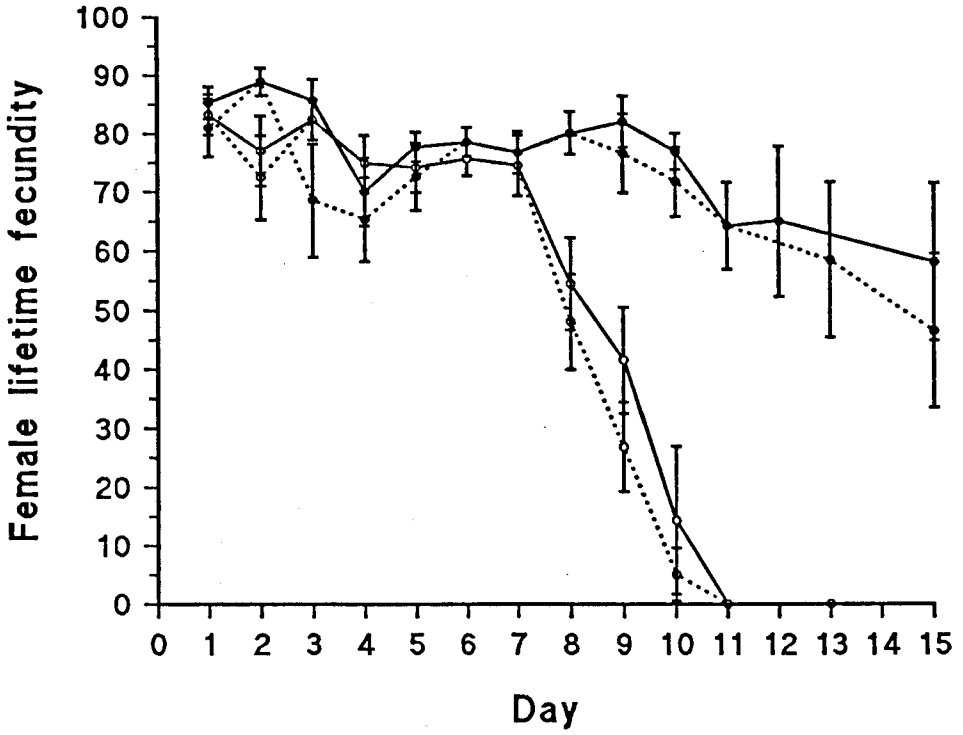

Fig. 5.2 The fertility of males able to both court and copulate throughout their lives (open circles) and hitherto virgin males (solid circles) either including all copulation opportunities, irrespective of whether copulation took place or produced viable offspring (dotted line) or including fertile copulations only (solid line).

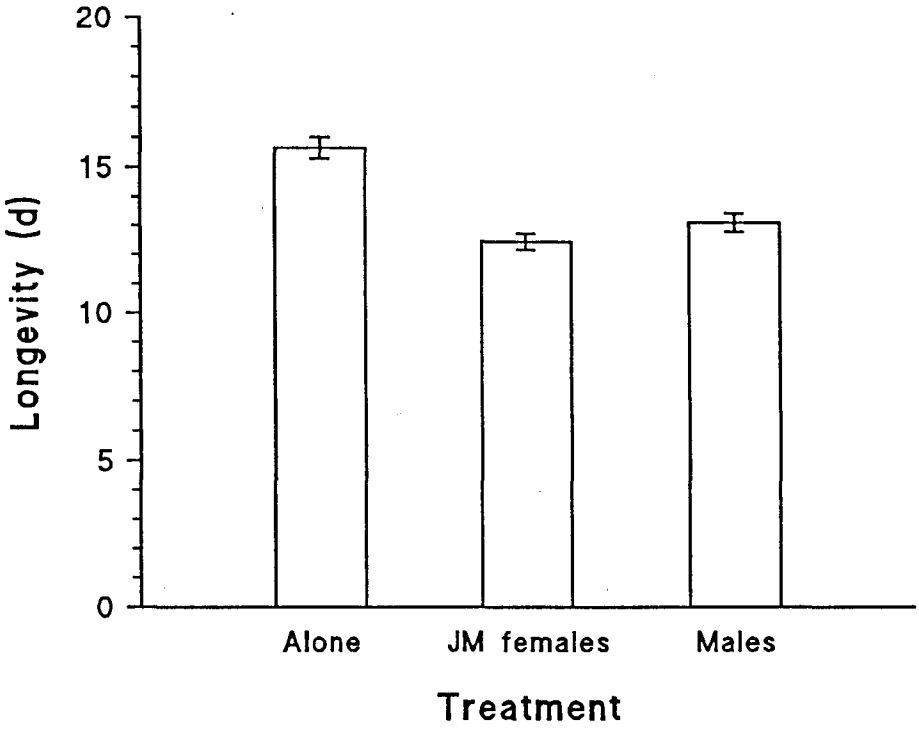

Fig. 5.3 The longevities of males kept alone (Group 1), able to court just-mated females (Group 2) or given access to with males (Group 3). Treatment mean longevities differed significantly (Kruskall-Wallis ANOVA: Chi-squared = 21.9, n = 44, $p < 0.001$). There was no significant difference between males given access to just-mated females or males (Mann-Whitney U-test, $U = 81.0$, $p > 0.05$)

Table 5.1 The mass of successive ejaculates.

Ejaculate mass was calculated by subtracting the change in mass measured in unmated control females from the change in mass measured in similar mated females.

	Mean change in mass of female (μg) ± s.d. (n)		Estimated ejaculate mass (μg)
	Mated female	Control female	
First ejaculate	208.1 ± 41.0 (12)	- 19.3 ± 19.9 (15)	227
Second ejaculate	113.4 ± 30.2 (9)	- 20.5 ± 25.8 (15)	134
Third ejaculate	47.6 ± 18.1 (7)	- 26.7 ± 47.5 (15)	74

5.3.3. The effects of repeated copulation

If males copulate repeatedly the number of fertile eggs resulting from each successive copulation declines until after six successive copulations further copulation by the male results in no fertile eggs (Fig. 5.4). This effect is apparent even when females that lay no eggs, who may not have received an ejaculate, are excluded from the analysis (Fig. 5.4). Male size, as indicated by elytral length, had no significant effect on either the number of fertile copulations (Spearman rank correlation: $r_S = -0.122$, $n = 18$, $p > 0.1$), the total number of copulations (Spearman rank correlation: $r_S = -0.145$, $n = 18$, $p > 0.1$) or the total number of hatching offspring from the four hours' sequence of copulations (Spearman rank correlation: $r_S = 0.020$, $n = 18$, $p > 0.1$). A few females stopped ovipositing for a day or more and then recommenced. This was more frequent in females mated to males which had mated more times previously (Fig. 5.5).

Twenty hours after the end of their exposure to the females males completely recovered their ability to fertilise females (t-test comparing the mean lifetime fecundities of the first females mated to each male in each bout: $t_{38} = 0.54$, $p > 0.1$) (Fig. 5.6). However, their fertility or ability to transfer ejaculate declines faster than previously unmated males of the same age (Mann-Whitney U-test comparing the mean lifetime fecundities of the fourth females mated to males which were either previously unmated or had mated up to 6 times 20 h before: $U = 9.00$, $n = 10$, $m = 8$, $p < 0.01$).

5.4. Discussion

The cost of reproduction in males is caused by copulation rather than courtship or competitive interactions with females. Copulation reduces the longevity of males and the size of subsequent ejaculates. The amount by which a copulation reduces the fertility of subsequent ejaculates depends on the interval between successive copulations; the reduction is greater if the interval is shorter. This suggests that males are able to replenish their supply of ejaculate material and that some of the costs of reproduction are transient.

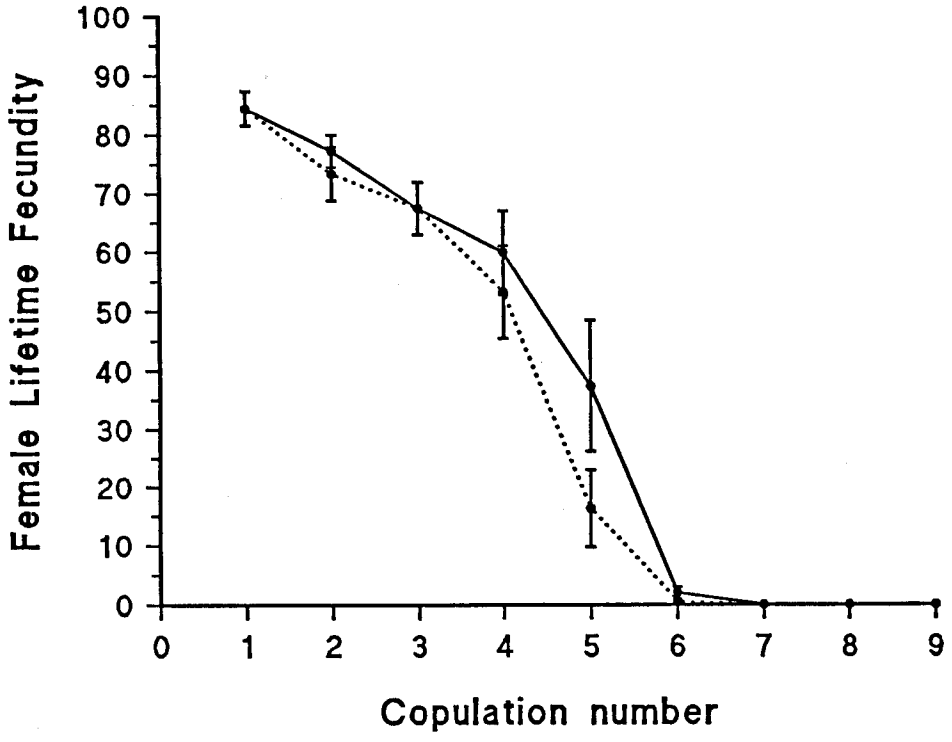

Fig. 5.4 The fertility of successive ejaculates of previously unmated males remating within a four hour period. Values are mean lifetime fecundities of females mated to these males either including infertile copulations (dotted line) or excluding infertile copulations (solid line). Errors are standard errors of the mean.

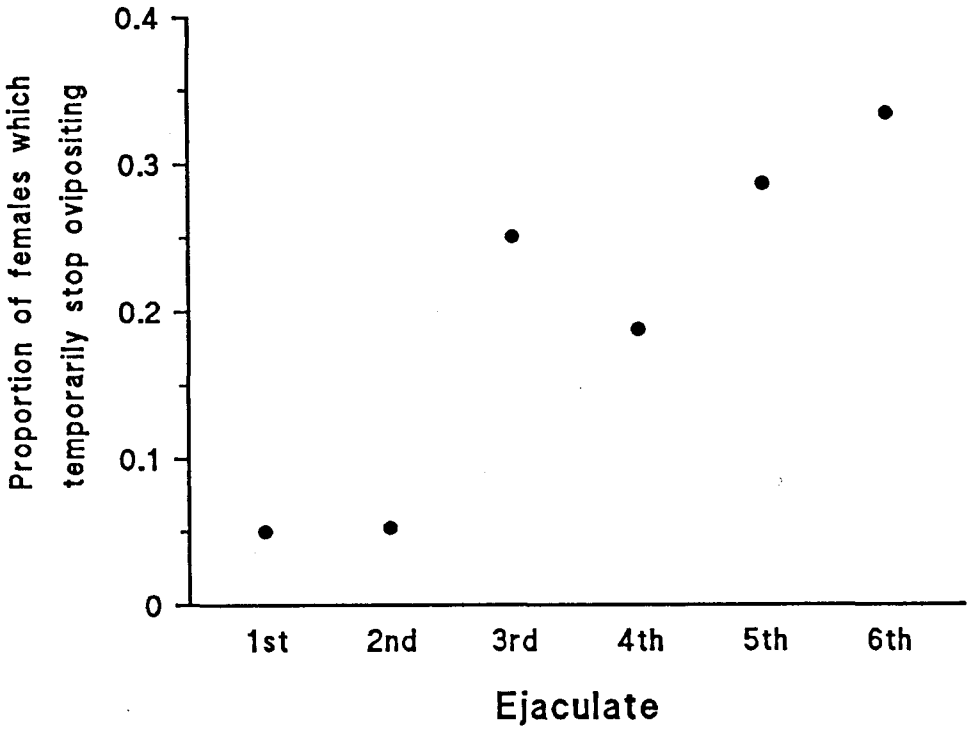

Fig. 5.5 The relationship between the probability that the female will temporarily stop ovipositing and the ejaculate's position in the sequence of successive copulations by the male (Spearman rank correlation: $r_s = 0.943$, $n = 6$, $p < 0.05$).

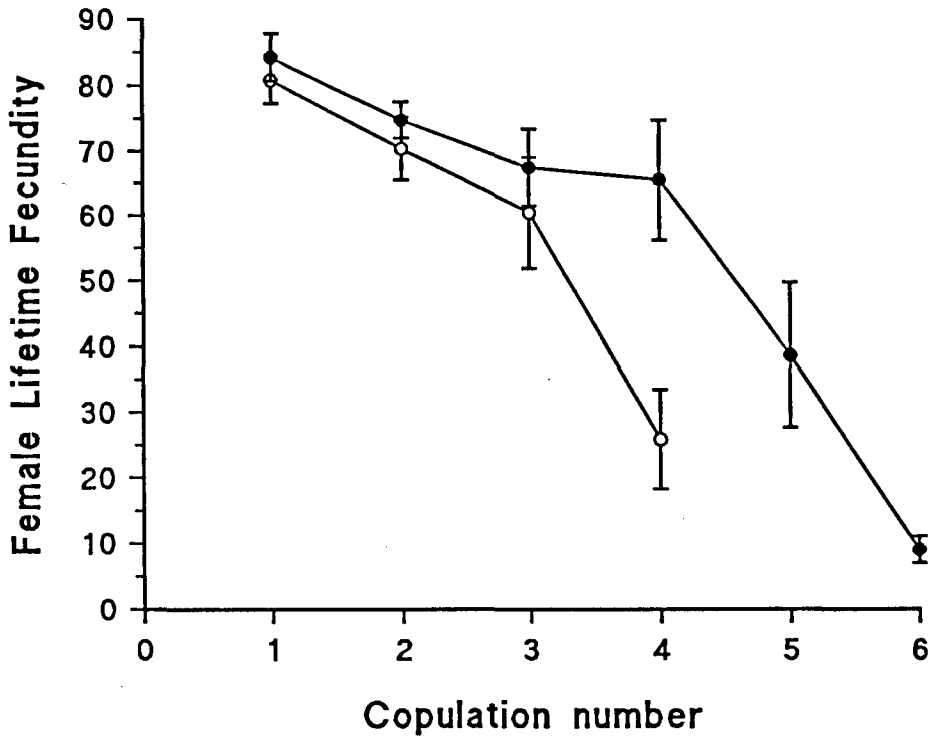

Fig. 5.6 The fertility of successive ejaculates males remating within a 4 h period. Males were either previously unmated (solid circles) or had mated up to 6 times 20 h before (open circles). Values are mean fecundities of females mated to these males excluding infertile copulations. Errors are standard errors of the mean.

5.4.1. Causes of the cost of reproduction

Males which both court and copulate die at an age while males which have not previously been exposed to females are still fully fertile. Males allowed to court unreceptive females also die sooner than males kept alone since emergence. However, because there is no difference between the longevities of males which are allowed to court but not copulate and of males which are kept with other males, the reduction in the longevity of courting males is probably a consequence of competition with females.

To ensure that the 'courting and copulating' males had similar levels of courtship to the 'courting' males, they were provided with females which could be courted but would not copulate. However, because just-mated females respond less to male courtship (Diptera: Leopold *et al.*, 1971; Fowler, 1973; Ramalingam and Craig, 1976. Orthoptera: Loher and Huber, 1966; Lynam *et al.*, 1992. Lepidoptera: Labine, 1964; Obara *et al.*, 1975; Sugawara, 1979), they may have provoked a lower level of courtship. In spite of attempts to minimise this problem by keeping the copulating males with just-mated females for the majority of their exposure to females, the males placed with virgin females may have suffered greater courtship costs than males placed with just-mated females.

However, the importance of copulation in causing the male cost of reproduction is likely to have been underestimated. Copulations in rapid succession are likely to be less costly to the male because they invest smaller amounts of ejaculate. The importance of a single copulation may be greater than the mean effect of a given number of successive copulations.

5.4.2. Payment of the costs of reproduction

i. Securing copulations

Two observations suggest that previously mated males might have had difficulty in courting and inseminating females. First, following copulation a male retracted his adeagus into his body; it took longer for him to do this when he had just mated several times (pers. obs). It is

possible that this was due to damage to the adeagus and, if so, that this damage hindered successful copulation. Second, towards the end of their lives, males in the 'courting and copulating' group were unable to court females adequately; they were unable to move either their hind legs or their antennae (pers. obs.). During a normal courtship ritual males vigorously tap the female's elytra with their antennae (Rup, 1986). Males with damaged antennae are less likely to obtain copulations with a female (P. Eady, pers. comm.). It would be interesting to investigate the relationship between a male's ability to antennate a female and his ability to produce a fertile ejaculate; is antennation by the male a means by which females assess male fertility?

ii. Producing a fertile ejaculate

The simplest explanation for the decline in mean fecundity of females mated to 'courting and copulating' males after the eighth day or to 'rapidly copulating' males after their fifth copulation is that the ejaculates they receive contain fewer sperm. This decline was observed even when analysis excluded copulations which produced no fertile offspring. It is not, therefore, due to the mean fecundity of these females being reduced by females which copulated but did not receive sperm.

The decline in male fertility may be due either to a reduced number of sperm inseminated or to sperm deterioration. Sperm number is known to decline with successive matings in male *C. maculatus* (Eady, 1992); this is consistent with the lack of sperm hypothesis. However, the decline in ejaculate mass and volume (pers. obs.) is consistent with a decline in any role non-sperm components may have in protecting the sperm from being damaged either during copulation or in the reproductive tract (*e.g.* Landa, 1960). Other ways in which ejaculate volume might contribute to the fertility of an ejaculate have been suggested; for example the volume of the spermatophore may transmit pressure from the walls of the female reproductive tract responsible for moving sperm along the reproductive tract (Gerber *et al.*, 1971). While plausible, these mechanisms remain unsubstantiated.

iii. Producing a manipulative ejaculate

While they remain fully fertile, changes in the size or the composition of successive ejaculates have no major effect on the female's oviposition schedule. However, it is impossible to distinguish between the effects of manipulation by 'hormonal' factors in the ejaculate and the declining sperm number. A lack of egg maturation or oviposition stimulants from the male could explain the larger proportion of females receiving a later ejaculate who temporarily stop ovipositing. However, this could also be due to the lower fertility of later ejaculates for one of two reasons. First, females laying fewer eggs have a higher probability of laying none on a given day by chance. Second, if there are costs associated with fertilising eggs with poor quality sperm, females inseminated with later ejaculates might temporarily retain eggs in anticipation of future copulations.

It is possible that ejaculate size, rather than ejaculate composition, has a role in manipulating subsequent female reproductive behaviour. Females receiving later ejaculates from a male mating three times successively have a shorter refractory period (Eady, 1992). The results of this chapter suggest that these ejaculates would have been fully fertile but smaller. The reasons for remating by females and the disadvantages of remating with a full bursa copulatrix are discussed in Chapter 6.

iv. Producing an ejaculate provides nutrients for the female

Females may gain a nutritional benefit from the ejaculate (see Chapter 6). The nutritional value of the ejaculate would be influenced by either its size or its composition. The decline in the size of successive ejaculates, if they had a nutritional role, could account for some of the decline in fecundity. However, the male ejaculate could only represent a small proportion of the mass of eggs produced by the female (the ratio of first ejaculate fresh weight to the fresh weight of a mean lifetime investment of 91 eggs is 1:10); especially after the male had mated several times previously and the ejaculate was smaller. However, the size of an ejaculate is not necessarily related to its nutritional value. For instance, Marshall and McNeil (1989) found no relationship between the spermatophore mass of the noctuid moth *Pseudaletia unipuncta* and the total lipid or hydrocarbon content of these spermatophores. The most likely situation in

which a nutritional role of this male ejaculate could account for such a marked change in the fecundity of females is if the ejaculate contained trace nutrients to which the female had no other access (*cf.* Schal and Bell, 1982; Pivnick and McNeil, 1987). However, male and female *C. maculatus* share the same source of nutrients as larvae , the black-eyed bean; they are unable to feed as adults. It is therefore unlikely that the decline in fecundity of successive ejaculates is related to their declining nutrient content.

5.4.3. Why do males continue to mate for little or no reproductive benefit?

Both the 'courting and copulating' males and the 'rapidly remating' males continued to mate even when they gained no reproductive benefit from mating. Their ejaculates may have contained no sperm or sperm may not have been transferred to the female successfully during copulation. One might expect males not to attempt to mate if mating were costly and they gained no offspring from the mating. Naively, it could be argued that they were behaving as though there were no cost of reproduction. However, there are several factors which may account for the apparent disregard males have for the cost of reproduction:

1) **The costs of reproduction for males transferring small ejaculates are low.** The cost of remating to a male transferring a small ejaculate is low in terms of the amount of resources invested.

2) **A rapidly remating male is unlikely to encounter sperm competition and hence a small ejaculate is less disadvantageous.** Ejaculate size is an important factor in determining the outcome of sperm competition. Therefore, there is a cost of transferring a small ejaculate because if the female remates, the male will not fertilise as many offspring as he would have if he had inseminated the female with a larger ejaculate. However, males will only get the opportunity to remate often enough to exhaust sperm when other males are rare and hence when females are unlikely to remate. Thus, males will only get the opportunity to exhaust their supplies of ejaculate when the costs of transferring a small ejaculate are reduced.

3) **Sperm-depleted males may still benefit from remating.** A male may reduce the amount of larval competition faced by his offspring if he delays fertilisation of the female or reduces her fecundity either by damaging her or by removing previous ejaculates. However, this hypothesis relies on the selection of spiteful behaviour and, in general, spite is not a stable strategy (Hamilton, 1970).

4) **The benefit to be gained from not mating may be low.** A male may have the opportunity to remate when he can inseminate few sperm. A larger ejaculate may result in an increased reproductive benefit from a copulation. However, there are diminishing reproductive benefits from additional ejaculate investment (Figs. 5.7 and 5.8). A male should mate whenever he has the opportunity, rather than waiting until his ejaculate supply is completely replenished, because he would get a greater reproductive benefit per unit of ejaculate invested. Moreover, he may be unlikely to gain another mating opportunity before he has the opportunity to completely replenish his ejaculate, even if he does mate now. In this case, there would be no benefit to be gained by not mating, in terms of an increasing the size of a subsequent ejaculate.

5) **The cost to a male of assessing his fertility and hence of determining whether he should mate or not may be high.** The later ejaculates of rapidly remating males occasionally result in a few fertile eggs. If males were to save the cost of infertile matings, they would need to assess their fertility. Inaccurate mechanisms for assessing fertility would result in the loss of offspring when the male behaved as though he were infertile when, in fact, he was not. The sensory mechanisms needed to assess fertility accurately may be prohibitively expensive.

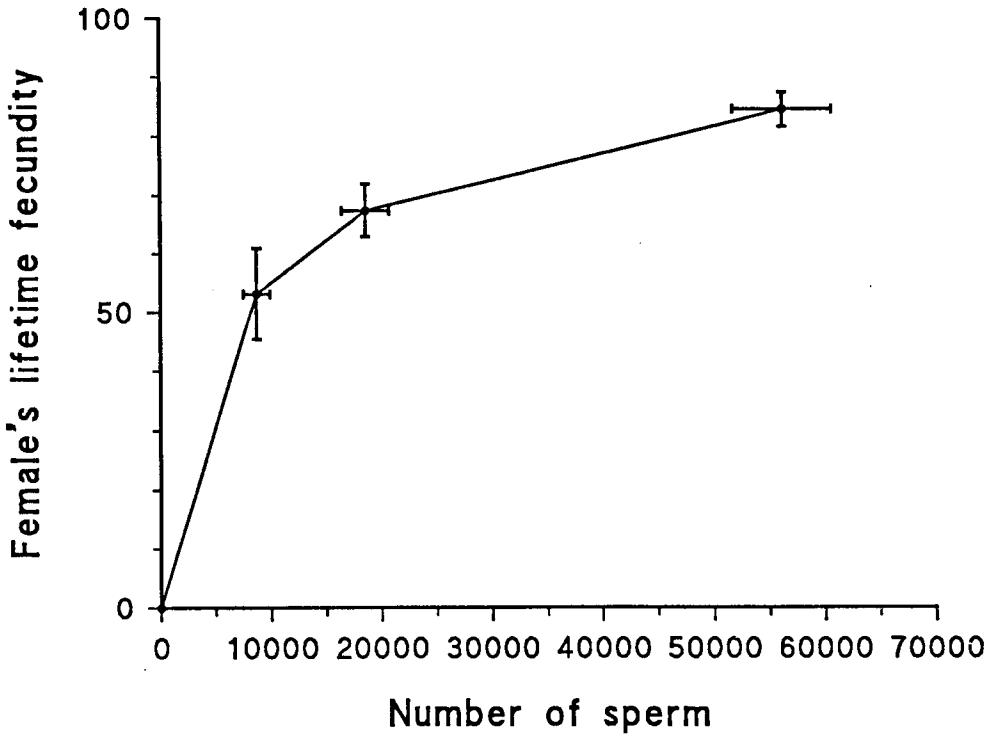

Fig. 5.7 The reproductive benefit to males of inseminating virgin females with different numbers of sperm. Values are the mean fecundities of females inseminated with ejaculates which differed in their sperm content. The number of sperm inseminated in successive ejaculates was measured by Eady (1992), the reproductive benefits from successive ejaculates were measured in 5.2.3. Errors are standard errors of the mean.

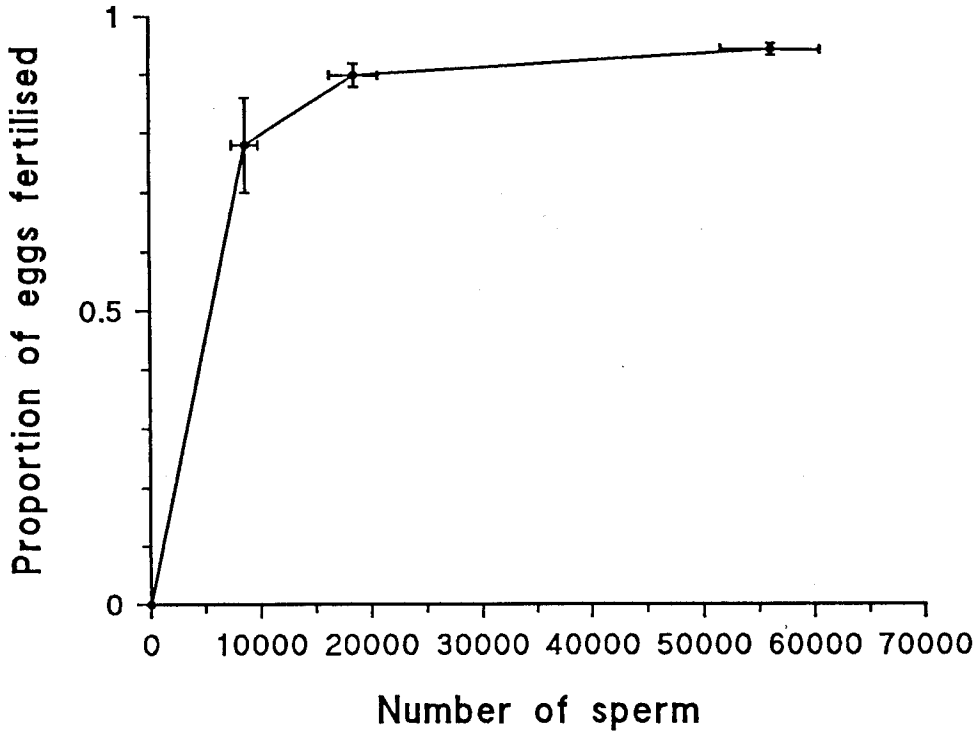

Fig. 5.8 The reproductive benefit to males of inseminating different numbers of sperm into females which had mated 48 h previously. Values are expressed as the proportion of fertile eggs oviposited after remating which were fertilised by the second male. The number of sperm inseminated in successive ejaculates and data on reproductive benefits from different sized ejaculates are from Eady (1992, pers. comm.). Errors are standard errors of the mean.

6. The costs and benefits of remating

6.1. Introduction

The cost of reproduction for females which have copulated only once has been discussed in previous chapters. However, females are not normally restricted to a single copulation. This chapter examines the costs of courtship and copulation. Why, if remating exposes females to the costs of courtship and copulation a second time, do they remate? It is possible that the costs are trivial or that females remate because they are forced to by males. However, they may benefit from remating. A number of potential benefits of remating are investigated. In particular the question of whether females can obtain a nutritional benefit from remating is addressed.

6.1.1. Costs of remating.

i. Copulation

Copulation may involve considerable costs to the female. Pathogen transmission can doubtless occur throughout courtship but is most likely to occur during copulation. Bacteria and viruses have been isolated from the ejaculates of several insects (Afzelius*et al.*, 1989; Kellen *et al.*, 1981); the importance to female insects of avoiding these pathogens is illustrated by the recent isolation of an antibacterial component in the secretions of the reproductive accessory glands of the female Medfly *Ceratitis capitata* (Marchini *et al.*, 1991).

Damage to the reproductive tract may occur either if it cannot withstand the mechanical stresses of copulation (Lloyd and Park, 1962; Oberhauser, 1989) or if males deliberately damage or obstruct it to prevent or delay remating, thus reducing sperm competition and maximising their paternity of the female's offspring (Labine, 1964; Parker, 1970; Drummond, 1984; Dickinson and Rutowski, 1989; Matsumoto and Suzuki, 1992).

ii. Manipulation by males

Males manipulate females in order to increase their own fitness, possibly at the expense of the female's fitness. In species where females remate, the reproductive benefit of a copulation to a male depends on the number of eggs a female is likely to fertilise with his sperm as opposed to the sperm of any other male. The male may be able to increase substantially his paternity of the females offspring by reducing the possibility that she fertilises her eggs with the sperm of other males (Parker, 1970; Birkhead and Hunter, 1990). Mechanisms by which the male maximises his paternity may be beneficial to the male even if they reduce the overall fecundity of the female; the expected gain in terms of eggs fertilised would have to outweigh the cost in terms of reduced female fecundity. For example, removal or damage of a previous fertile ejaculate by a second male may involve some cost to the female in terms of physical damage, yet is obviously of great benefit to this male.

If a male stimulates a female to increase her oviposition rate following copulation, this may increase the rate at which she fertilises eggs using his sperm. This will reduce the number of eggs he risks losing should the female remate with another male after a given time. While this male-mediated short term increase in oviposition rate may increase female lifetime fecundity under laboratory conditions, an increased oviposition rate may not be the optimal female strategy if either the availability or quality of oviposition sites are unpredictable (*e.g.* Mark, 1982), *i.e.* if restraint or prolonged oviposition site assessment are beneficial (Nwanze and Horber, 1976; Mitchell, 1983; Messina and Renwick, 1985; Wilson, 1988; Thanthianga and Mitchell, 1990) or if it is better to retain eggs when oviposition sites are of poor quality for later oviposition on pristine seeds. Thus, an apparent remating benefit for females in the laboratory may not represent a benefit in the natural environment.

6.1.2. Benefits of remating

i. Benefit from the ejaculate itself

1) The ejaculate may provide nutrients; the uptake of male-derived material and its incorporation into both female tissues and oocytes have been demonstrated in many insects (review: Parker and Simmons, 1989), including the bruchids *Caryedon serratus* (Boucher and Huignard, 1987) and *Acanthoscelides obtectus* (Huignard, 1983).

2) The second ejaculate may replace an ejaculate of low fertility; the first ejaculate might have been of low fertility when passed from the male, or have declined in fertility by the time it would be used to fertilise eggs (Taylor, 1967; Martin et al., 1989; Danthanarayana and Gu, 1991; Petersson, 1991). Ejaculate fertility is affected by the 'quality' of both sperm and accessory material which are thought to provide optimal conditions for sperm survival and/or function (nutrients: Chen 1984; enzymes: Gilbert, 1981; ionic composition: Khan and Musgrave, 1969).

3) The second ejaculate might flush out an ejaculate infected with pathogens.

4) The female might benefit by fertilising her eggs with genetically disparate sperm or by choosing between ejaculates for genetically superior sperm (Williams, 1975; Parker, 1984; Watson, 1991).

ii. Indirect benefit from remating

Harassment by males may prevent females from ovipositing. Males may be less likely to harass the female allowing her to oviposit if they have mated with her. If this is so, one might find females which mate to avoid harassment (Parker, 1970b).

6.1.3. Nutritional benefit from remating.

Demonstrating a nutritional role for the ejaculate is difficult. While the spermatophores of *C. maculatus* are relatively large (4% of male emergence weight, section 5.3.3), the argument that spermatophore size is related to any nutritional function (*e.g.* Ridley, 1988) is invalid without some knowledge of spermatophore composition and female nutritional requirements (Marshall, 1982; Bownes and Partridge, 1987; Marshall and McNeil, 1989). The demonstration that females remating with males that produce smaller ejaculates have a lower fecundity (Rutowski *et al.*, 1987) does not neccessarily imply a nutritional function; the ejaculate quality of poor-quality males may limit female fecundity if sperm quality or maintenance is reduced due to, say, accessory gland depletion (Hihara, 1981) (section 6.1.2).

A demonstration that spermatophores are digested is not sufficient to imply a nutritional role. Spermatophores may be digested for a number of reasons other than for a nutritional benefit: they may be digested to avoid congestion of the reproductive tract and allow remating or oviposition, or simply as a consequence of hostile conditions which prevail within the female reproductive tract to prevent infection. If the spermatophore, for whatever reason, is digested one might expect subsequent uptake of metabolites even if their nutritional role were trivial. Thus, the demonstration that male-derived nutrient is incorporated into insect oocytes or into the female (Boggs and Gilbert, 1979; Friedel and Gillott, 1977; Bowen *et al.*, 1984) does not imply a major nutritional role for the ejaculate.

It has been suggested that the only way to show unequivocally that the ejaculate has a nutritional role is to demonstrate a remating benefit in impoverished females which is reduced in well nourished females (Gwynne, 1984; Turner and Anderson, 1983). In these experiments, the only difference between females, if males are allocated randomly, is their nutritional requirements; the composition of inseminated ejaculates should not vary between groups. If impoverished females benefit more from remating than well resourced females, this must be due to nutritional differences between the two groups. It is argued that the lack of remating benefit is due to the irrelevance of nutrients contained in the ejaculate to well fed females. However, this argument is seriously flawed in insects which are adapted for an environment

in which food supply is variable. Under these conditions, a female with restricted food supply might benefit by withholding oviposition until her environment improves; the male ejaculate may contain ejaculate components which stimulate oviposition in these females. Conversely, a female with a good food supply may well be unaffected by these ejaculate components because she is already ovipositing at her maximal rate. Female *C. maculatus*, however, would not anticipate future opportunities to feed in grain stores; unfed females would not therefore be expected to oviposit at lower rates than fed females because they anticipated an improvement in their environment.

6.1.4. Aims

The aims of this chapter were:

1. To measure the costs to females of repeated copulation.

2. To measure the fitness consequences to females of less extreme remating schedules.

3. To investigate the aspect of remating which caused these fitness consequences.

6.2. Methods

6.2.1. The effects of repeated copulation

In an experiment to assess the costs or benefits of repeated copulation, virgin females, 1-18 h after emergence, and virgin males, 1-30 h after emergence, were randomly allocated to one of the following treatments:

Treatment 1: female + male

Treatment 2: female + 3 males

Treatment 3: female + 5 males

Beetles were placed in 25 ml containers and were checked at 12 h intervals to determine their longevity. Males which died before the female were replaced with younger non-virgin males.

After their death, the females' elytral lengths were measured and they were dissected to determine whether the reproductive tract was intact.

6.2.2. Remating by females of differing nutritional status

The fitness consequences of differing mating schedules were investigated in females which differed in their nutritional status. Nutritional status was manipulated by controlling larval competition and the period of time before females were first mated. The mating schedules of these females were then manipulated by restricting the availability of males.

Virgin females were obtained from two culture boxes which differed in larval density. The mean nutritional statuses of adults emerging in the two culture boxes were expected to differ; those which had experienced relatively low larval competition would be relatively well-resourced (Credland and Dick, 1987). The first of the females to emerge in the 'high larval intensity' culture box were isolated for five days prior to being mated. By the time that they were mated, they had metabolised some of their already limited supply of nutrients. Thus, three groups of females, differing in nutritional status were created:

Well-resourced females, which had suffered little larval competition and were mated for the first time soon after emergence.

Moderately-resourced females, which had suffered intense larval competition and were mated for the first time soon after emergence.

Poorly-resourced females, which had suffered intense larval competition and were mated 5 d after emergence.

The number of matings which could reliably be recorded at once was lower than the sample size needed for analysis. Consequently, each group was composed of two replicates so that the combined sample size was sufficient. The mean daily fecundities, longevities, elytral lengths and numbers of infertile eggs measured within groups differing in nutritional status and within mating treatment were compared between replicates using t-tests or Mann-Whitney U tests: 12 out of the 88 comparisons were significantly different at a level of 0.05,

more than would be expected by chance. Consequently data could not be combined; all statistical tests controlled for the effects of variation between replicates.

Each female was mated to a virgin male. This first mating took place within 1 d of emergence for the well-resourced and moderately-resourced females or between 5 and 6 d of emergence for the poorly-resourced females. Twenty of these once mated females were randomly allocated to treatment 1, females which would not mate again. The original intention was to allocate females randomly to treatments in which they mated on the first day only (1), the first and second days (12), the first second and third days (123) and on the first, second, third and fourth days (1234). However, because many females refused to mate on the second day, alternative treatments were used:

1: a random sample of females mating on day 1

12: females mated on day 1 and remated on day 2. This was not a random sample of the females mated on day 1; some females refused to mate.

13: females mated on day 1 which refused to remate on day 2 and then remated successfully on day 3

135: a random sample of 13 females which were remated successfully on day 5.

The experimental design (summarised in Fig. 6.1) allows comparison between once-mated and remated females. The validity of comparisons between groups is determined by whether females were randomly allocated between. Thus, the only valid comparison between individual groups is that between 13 and 135 females. Other comparisons are confounded by whether or not females remated on day 2.

The following traits were measured: lifetime fecundity, longevity, egg length, the proportion of eggs which were infertile, the proportion of offspring which emerged as adults and their developmental period. Because of the short developmental period, it was not possible to measure all traits for all females. Egg length was measured only for moderately-resourced females. Neither the proportion of offspring which emerged as adults nor their developmental period were measured for poorly-resourced females (Table 6.1).

					Manipulation of Mating schedule			
					1			
						12	1 not 2	
							13	135
Manipulation of resource status	Rep. 1	Low larval competition		w-r				
		Intense larval competition	not delayed	m-r				
			delayed	p-r				*
	Rep. 2	Low larval competition		w-r				
		Intense larval competition	not delayed	m-r				
			delayed	p-r				*

Fig. 6.1 Plan of the experiment described in 6.2.2. Females were allocated to one of three resource levels and then mated on the first day only (1), the first and second days (12), the first and third but not the second (13) and the first, third and fifth but not the second (135). The experiment was replicated. Poorly-resourced females did not live long enough for there to be poorly-resourced 135 groups (*).

Table 6.1 The life history traits measured in females of the different nutrient status and their offspring. Traits which were measured are indicated by an asterisk (see 6.2.2 for a description of experimental design).

	Well-resourced	Moderately-resourced	Poorly-resourced
Lifetime fecundity	*	*	*
Longevity	*	*	*
Egg length		*	
Proportion of eggs which were infertile	*	*	*
Developmental period of offspring	*	*	
Proportion of offspring which emerged as adults	*	*	

6.3. Results

6.3.1. Costs of repeated copulation

There was no difference between the mean elytral lengths of females in each of the three treatments (Kruskall-Wallis 1 way analysis of variance: chi-square = 1.99, d.f. = 2, $p > 0.1$). Females placed with three or more males died sooner than females placed with one male (Fig. 6.2) and more of them had ruptured reproductive tracts (Table 6.2).

There was no difference between the longevities of females with ruptured and intact reproductive tracts in treatment 1; however, females with ruptured reproductive tracts in both treatments 2 and 3 died sooner than intact females from the same treatment (Fig. 6.3). It is possible that a few of the reproductive tracts were ruptured during dissection; this could account for the similarity of longevities of ruptured and intact females in treatment 1. However, there is no reason to suppose that any more reproductive tracts would be damaged during dissection in treatments 2 and 3 than in treatment 1 so rupturing of reproductive tracts must be caused by the greater number of males in these treatments and presumably by more frequent copulation. There was no difference between the elytral lengths of females with ruptured and intact reproductive tracts when the data for all three treatments were combined. However, when each treatment was analysed separately, females placed with 5 males whose reproductive tracts remained intact were significantly larger than those whose reproductive tracts ruptured (Fig. 6.4)

6.3.2. Remating by females of differing nutritional status

i. The effects of the nutritional manipulations

The 'low larval competition' and 'intense larval competition' culture boxes contained beans with mean egg loads of 1.74 (s.e. = 1.01) eggs per bean and 10.0 (s.e. = 3.01) eggs per bean respectively. The well-resourced females were larger than the other two groups of females

Fig. 6.2 The longevities of females individually placed in 25 ml containers with 1, 3 or 5 virgin males (see 6.2.1) The treatment mean longevities differed significantly (ANOVA: $F_{2, 50} = 15.7$, $p < 0.001$).

Table 6.2 The frequencies with which females' reproductive tracts ruptured when they were placed in 25 ml containers with 1, 3 or 5 virgin males. Chi-square = 8.61, d.f. = 2, $p < 0.05$.

	Ruptured	Intact
One male	2	16
Three males	7	10
Five males	10	8

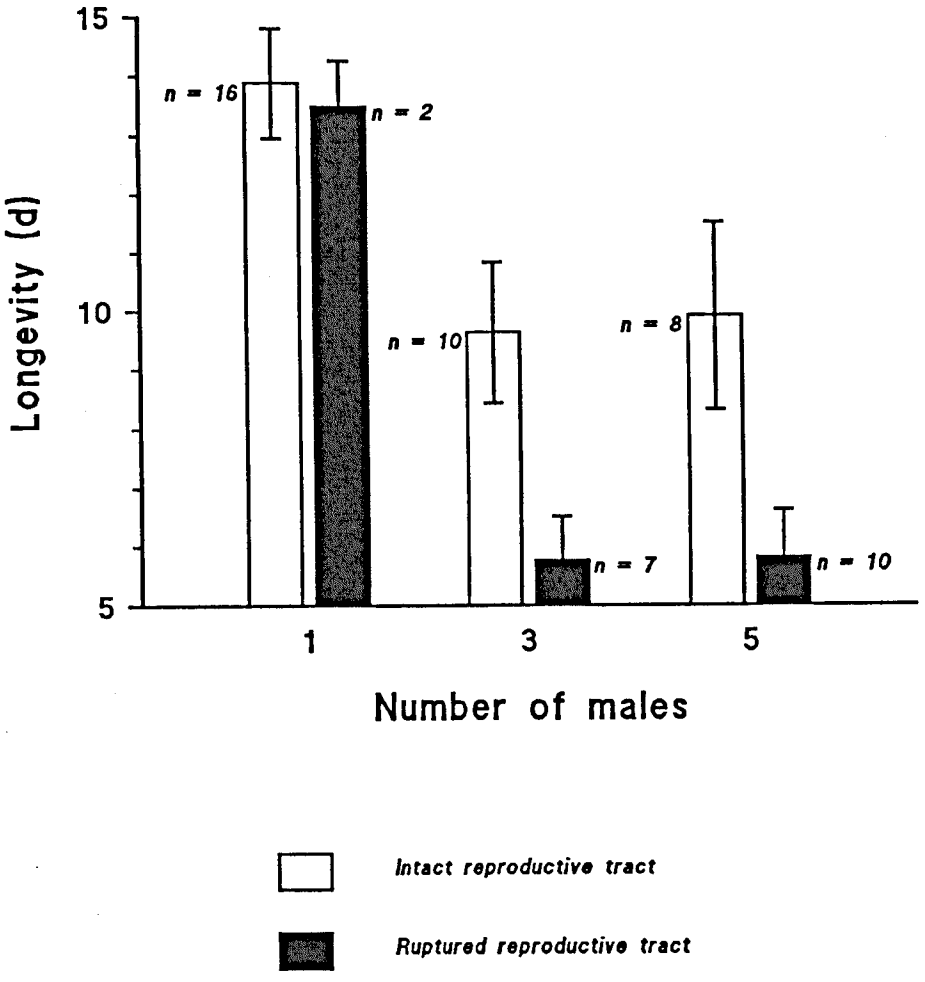

Fig. 6.3 The longevities of females individually placed in 25 ml containers with 1, 3 or 5 virgin males, distinguishing between females with ruptured reproductive tracts (solid bars) and intact' females (open bars) (see 6.2.1). The longevities of ruptured and intact females were significantly different (t-test, using combined data from all three treatments: $t = 4.46$, d.f. = 51, $p < 0.001$). When treatments were analysed separately, there were significant differences between the longevities of ruptured and intact females only when females were placed with more than one male (Mann-Whitney U tests corrected for ties: 1 male, $z = -0.849$, $n = 18$, $p > 0.05$; 3 males, $z = -2.548$, $n = 19$, $p < 0.05$; 5 males, $z = -2.275$, $n = 18$, $p < 0.05$); sample sizes were insufficient to perform a two-way analysis of variance.

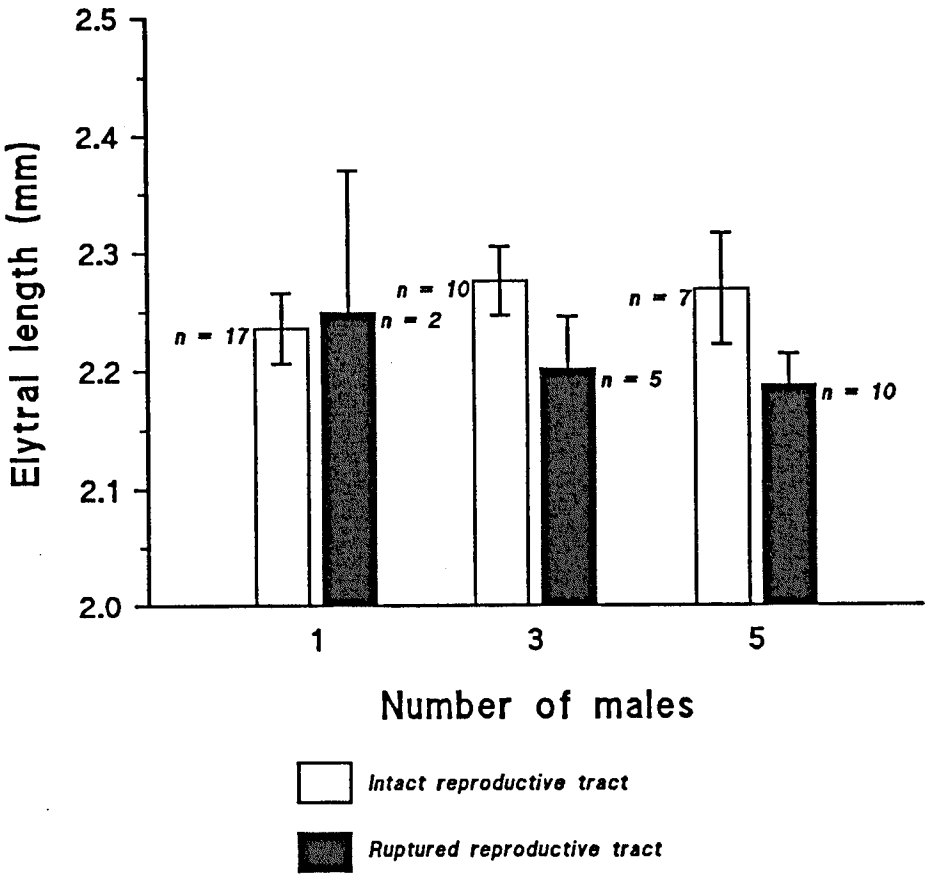

Fig. 6.4 The elytral lengths of females individually placed in 25 ml containers with 1, 3 or 5 virgin males, distinguishing between females with ruptured (solid bars) and intact (open bars) reproductive tracts (see 6.2.1). There was no significant difference between the elytral lengths of ruptured and intact females when data from all three treatments were combined (t-test: $p > 0.05$). When treatments were analysed separately, there were only significant differences between ruptured and intact females which had been placed with 5 males (Mann-Whitney U test corrected for ties: $z = -2.201$, $n = 17$, $p < 0.05$).

which were a similar size (Table 6.3). There were significant differences in the mean lifetime fecundities of once mated females with differing resource levels (Table 6.3).

ii. The effects of remating

In many groups there were significant increases in the rate of oviposition during the 24 h following remating (Table 6.4). These increases were not prolonged and the daily rate of oviposition subsequently fell to the level of 'once-mated' females (these daily rates of oviposition are shown for well-resourced, moderately-resourced and poorly-resourced females in Figs. 6.5 - 6.7 respectively). Nonetheless, in spite of the small sample sizes, these increases were sufficient to increase the lifetime fecundities of both well-resourced and moderately resourced females (Fig. 6.8). Other life history traits were not significantly affected by the remating schedule (Table 6.5, Figs. 6.9-6.16).

The increases in lifetime fecundity were still apparent when females with unusually low first day fecundities (more than one standard deviation below the mean first day fecundity for their treatment) were excluded from the analysis (ANOVAs: well-resourced females $F_{3,62} = 3.633$, $p < 0.018$; moderately-resourced females $F_{3,78} = 3.717$, $p < 0.015$).

iii. The effects of nutritional status

Nutritional status did not significantly affect the effects of remating on lifetime fecundity (Table 6.6).

6.4. Discussion

6.4.1. Costs of remating

Rupturing is a visible sign of damage to the reproductive tract and is presumably the result of repeated copulation. It seems likely that the reduced longevities of repeatedly mated females with intact reproductive tracts are due to less profound damage. Ruptured females are found

Table 6.3 The effect of nutritional status on mean female elytral length (data were combined from all treatments) and the mean lifetime fecundities of once-mated females (see 6.2.2). Errors for both traits are standard errors of the mean. Well-resourced females were significantly larger than other females as indicated by elytral length (ANOVA: $F_{2, 260} = 20.88$, $p < 0.001$). There were significant differences between the lifetime fecundities of once-mated females (ANOVA: $F_{2, 57} = 9.38$, $p < 0.001$).

	Elytral length (mm)	Lifetime fecundity of once mated females
Well-resourced	2.18 (0.009)	109.1 (4.30)
Moderately-resourced	2.10 (0.010)	100.1 (3.76)
Poorly-resourced	2.10 (0.012)	85.5 (3.57)

Table 6.4 The summarised effects of remating on life-history traits (see 6.2.2.). Asterisks indicate a significant effect of remating on the value of the life history trait (* , $p < 0.05$). n.s. indicates that there was no consistent effect. Dashes indicate that the trait in question was not measured (see text). Tests for lifetime fecundity and adult longevity were two way ANOVAs, controlling for the effects of differences between replicates. F-values for these tests are reported in Table 6.5. Mean values of egg length, the proportion of eggs infertile, the developmental period of offspring and the proportion of offspring which emerged as adults were calculated for each days oviposition, no consistent remating effects were observed (Figs. 6.9-6.16).

	Well-resourced	Moderately-resourced	Poorly-resourced
Lifetime fecundity	*	*	n.s.
Longevity	n.s.	n.s.	n.s.
Egg length	-	n.s.	-
Proportion of eggs which are infertile	n.s.	n.s.	n.s.
Developmental period of offspring	n.s.	n.s.	-
Proportion of offspring which emerge as adults	n.s.	n.s.	-

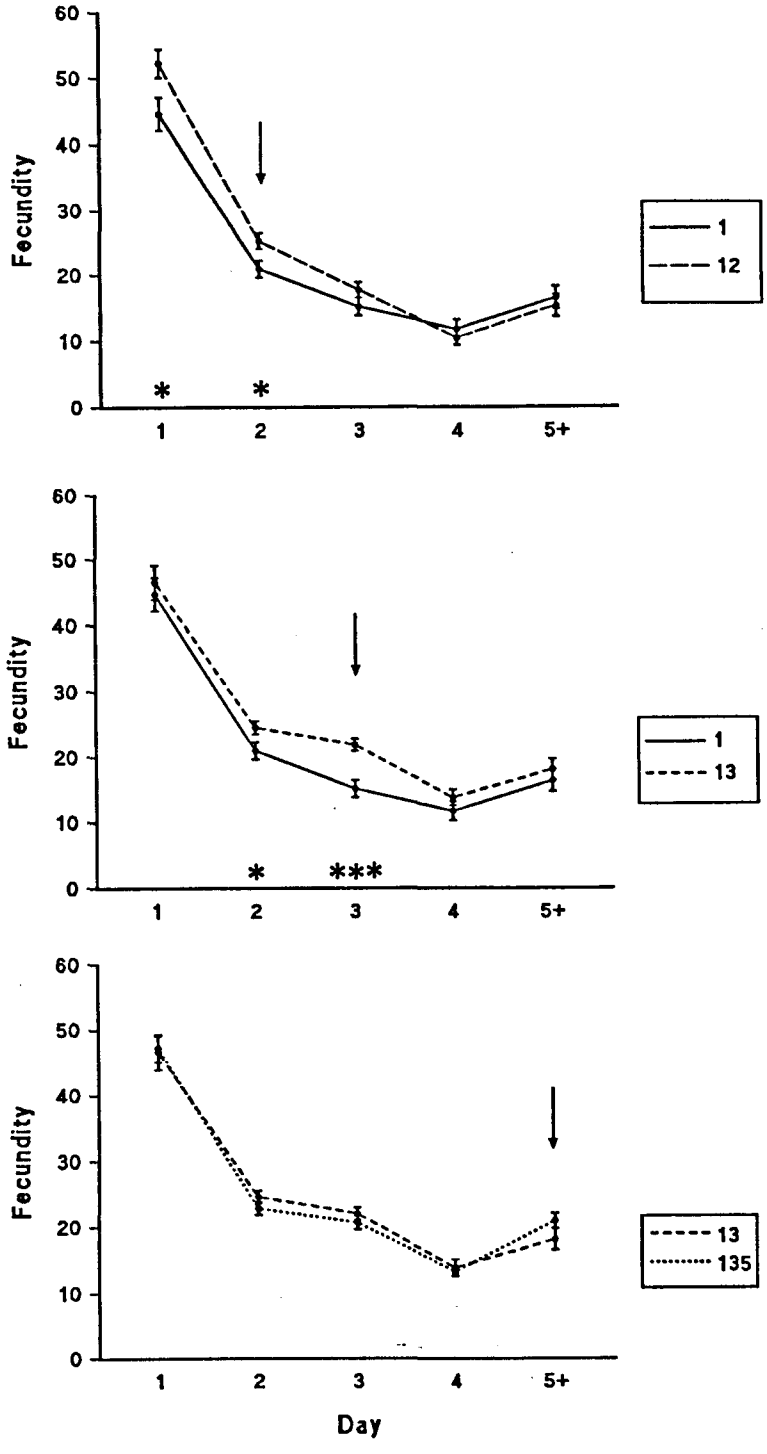

Fig. 6.5 The daily fecundities of well-resourced females with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other. Asterisked pairs of daily fecundities differed significantly (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). t-tests were used if the combined data from the two replicates were normally distributed. A Mann-Whitney U test was used when the combined data were not normally distributed.

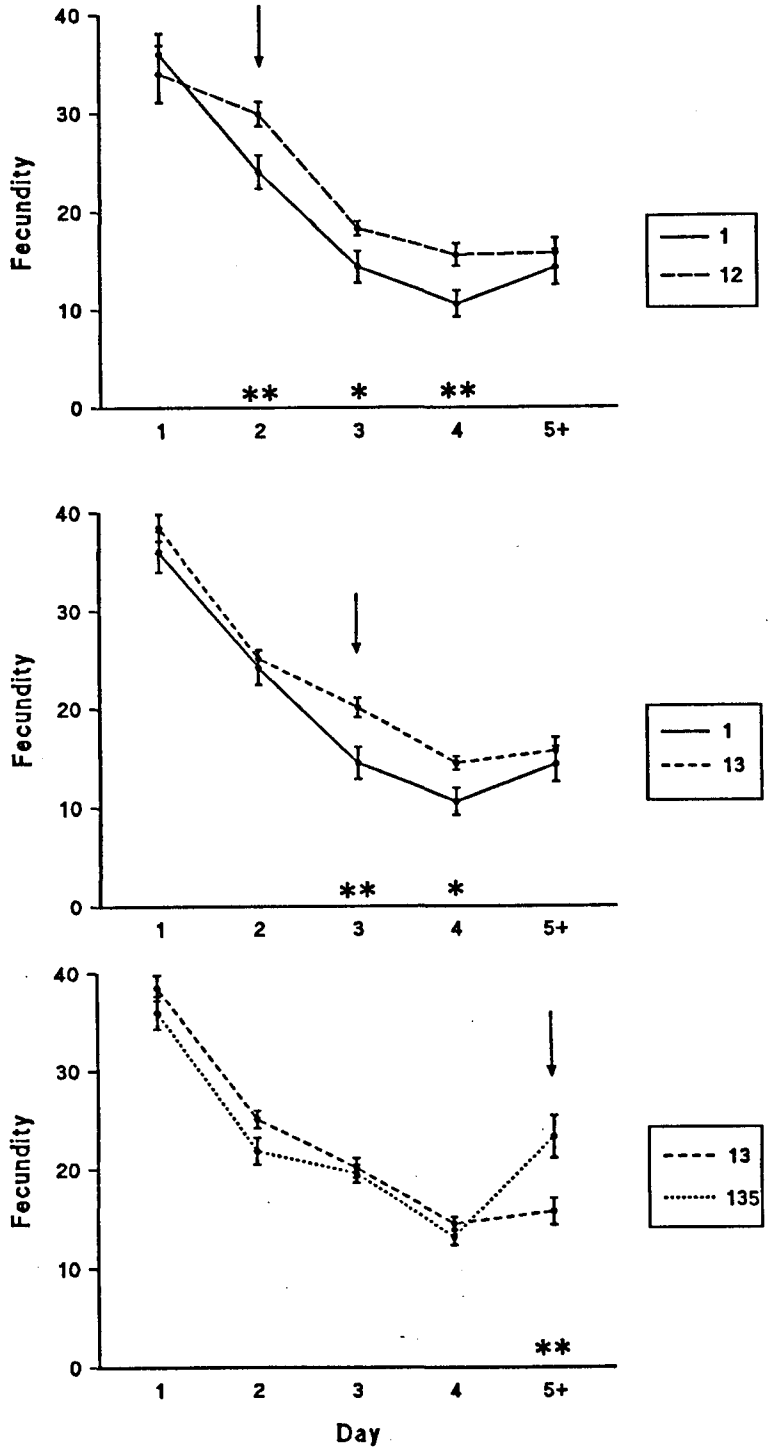

Fig. 6.6 The daily fecundities of moderately-resourced females with differing mating schedules (see 6.2.2. for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other. Asterisked pairs of daily fecundities differed significantly (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) when tested using t-tests or Mann-Whitney U tests as appropriate (see Fig. 6.5).

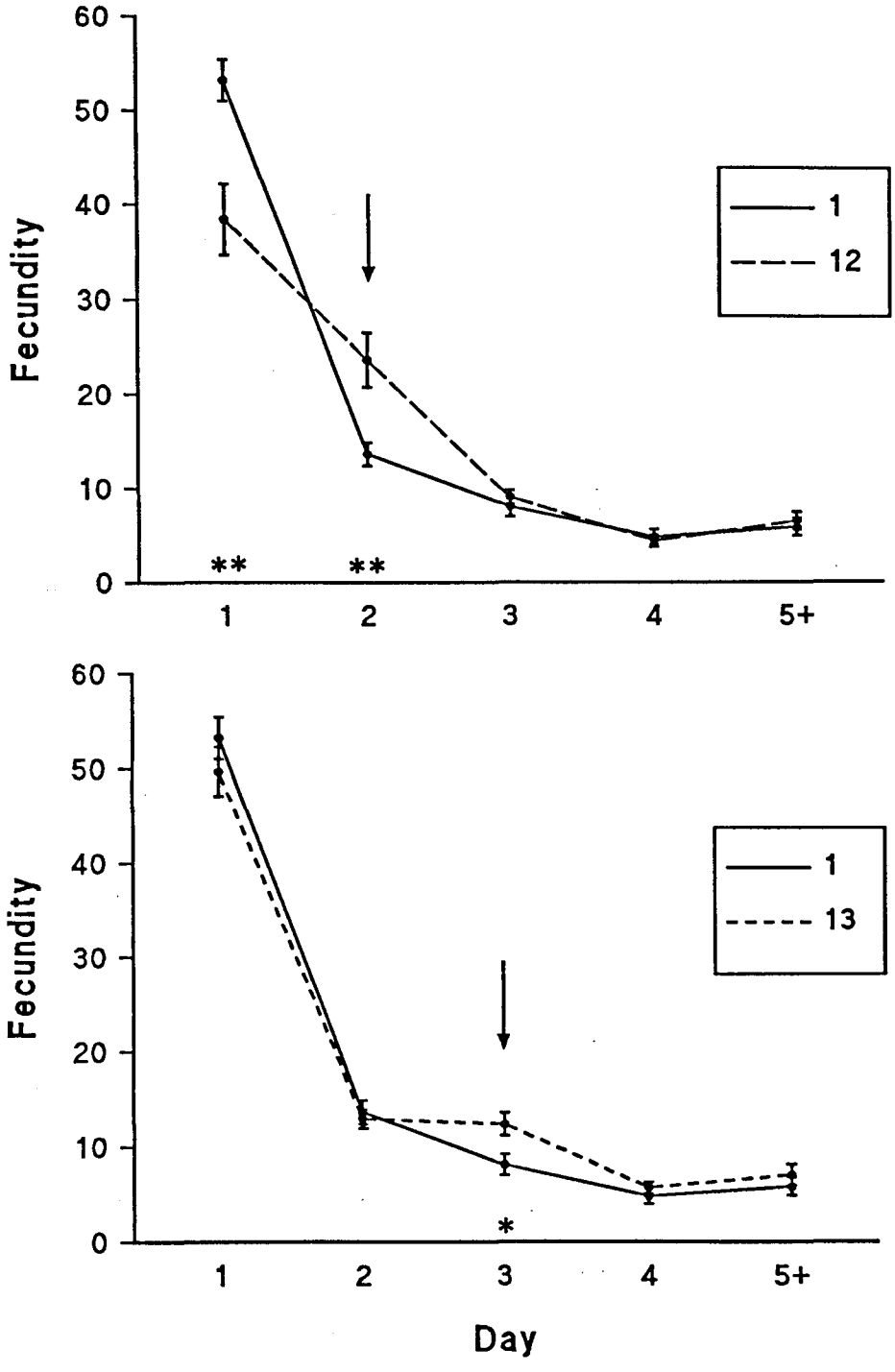

Fig. 6.7 The daily fecundities of poorly-resourced females with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other. Asterisked pairs of daily fecundities differed significantly (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) when tested using t-tests or Mann-Whitney U tests as appropriate (see Fig. 6.5).

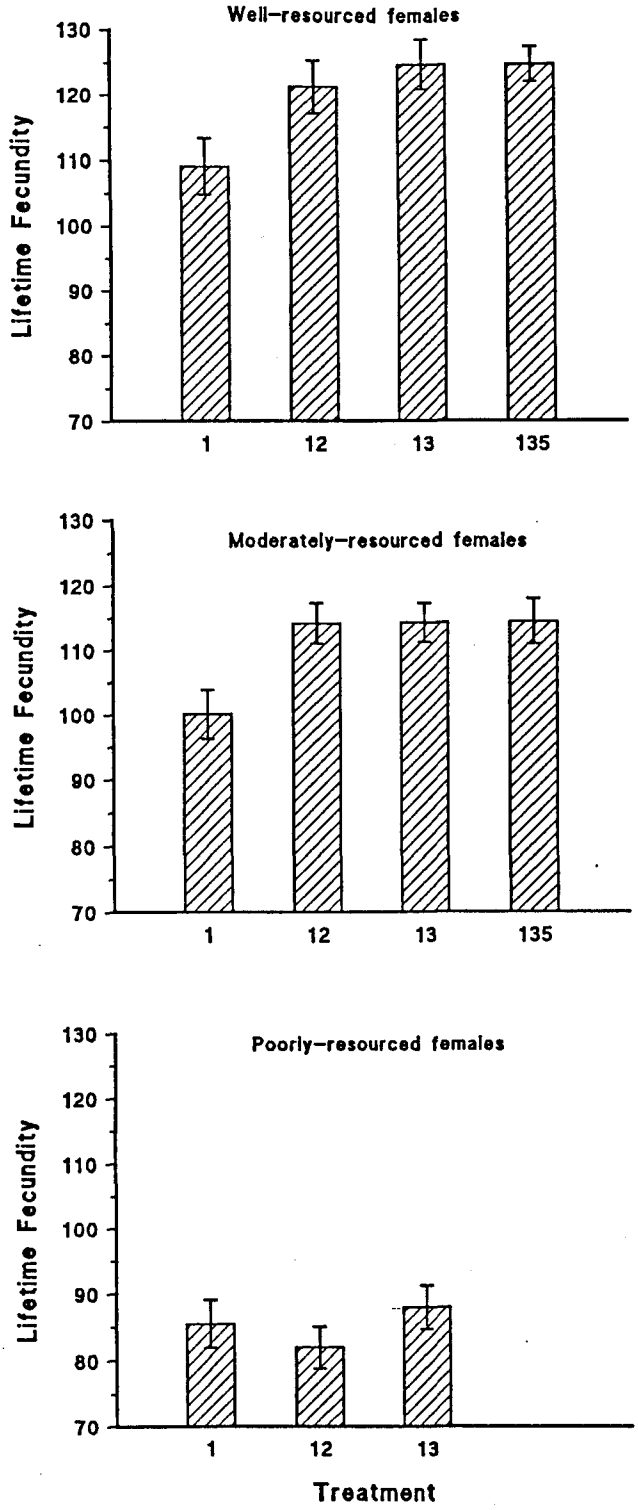

Fig. 6.8 The lifetime fecundities of females with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). There were significant increases following remating in the lifetime fecundities of well- and moderately- but not poorly-resourced females (two-way ANOVA, controlling for the effects of differences between replicates: well-resourced females, $F_{3, 76} = 3.753$, $p < 0.05$; moderately-resourced females, $F_{3, 88} = 3.403$, $p < 0.05$; poorly-resourced females, $F_{2, 63} = 0.806$, $p > 0.05$). *A posteori* comparisons using the Tukey-Kramer procedure revealed no significant differences between the lifetime fecundities of twice and thrice mated females ($p > 0.05$). The two-way ANOVAs showed no significant effect on the lifetime fecundities of either replicate or the interaction between replicate and remating schedule ($p > 0.05$)

Table 6.5 The summarised effects of remating on life history traits. F-values for the ANOVAs used to test for the effects of remating on the value of life history traits (see Table 6.4).

	d.f.	F	p
Well-resourced:			
Lifetime fecundity	3, 76	3.753	< 0.05
Longevity	3, 76	2.442	> 0.05
Moderately-resourced:			
Lifetime fecundity	3, 88	3.403	< 0.05
Longevity	3, 88	0.198	> 0.05
Poorly-resourced:			
Lifetime fecundity	2, 63	0.806	> 0.05
Longevity	2, 63	0.494	> 0.05

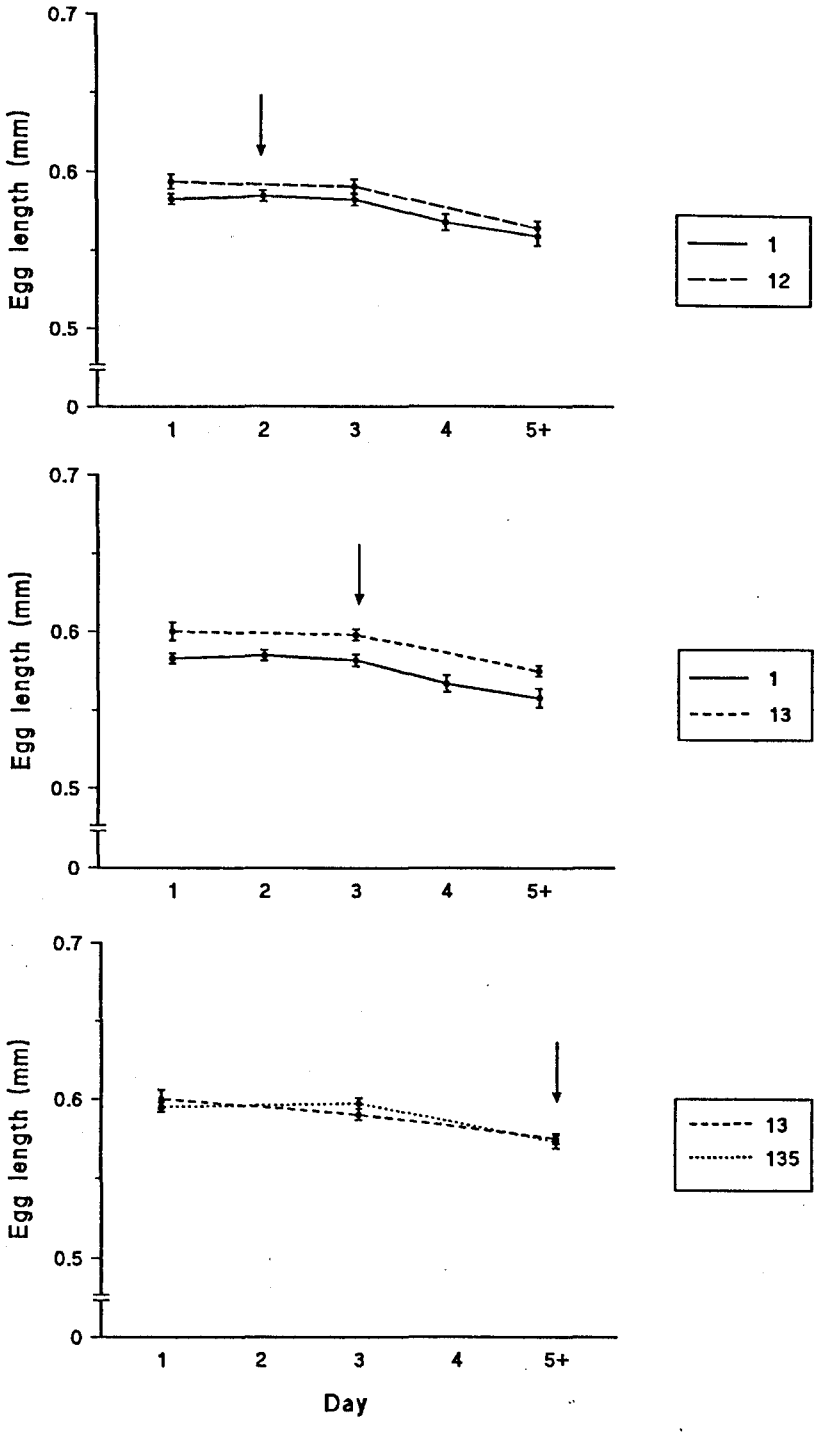

Fig. 6.9 The **mean length of eggs** laid on successive days by **moderately-resourced females** with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other.

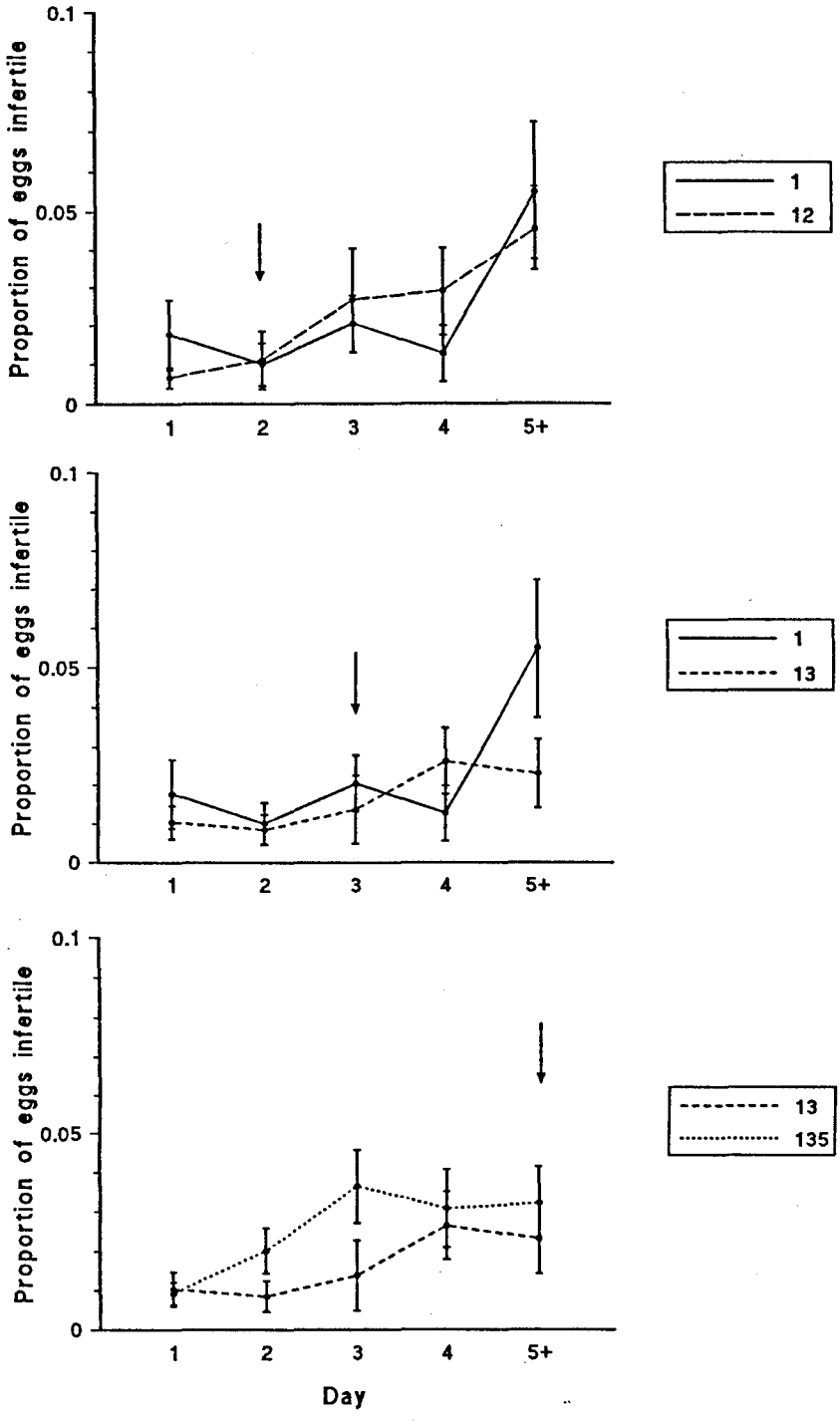

Fig. 6.10 The **proportion of eggs which were infertile,** oviposited on successive days by **well-resourced females** with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other.

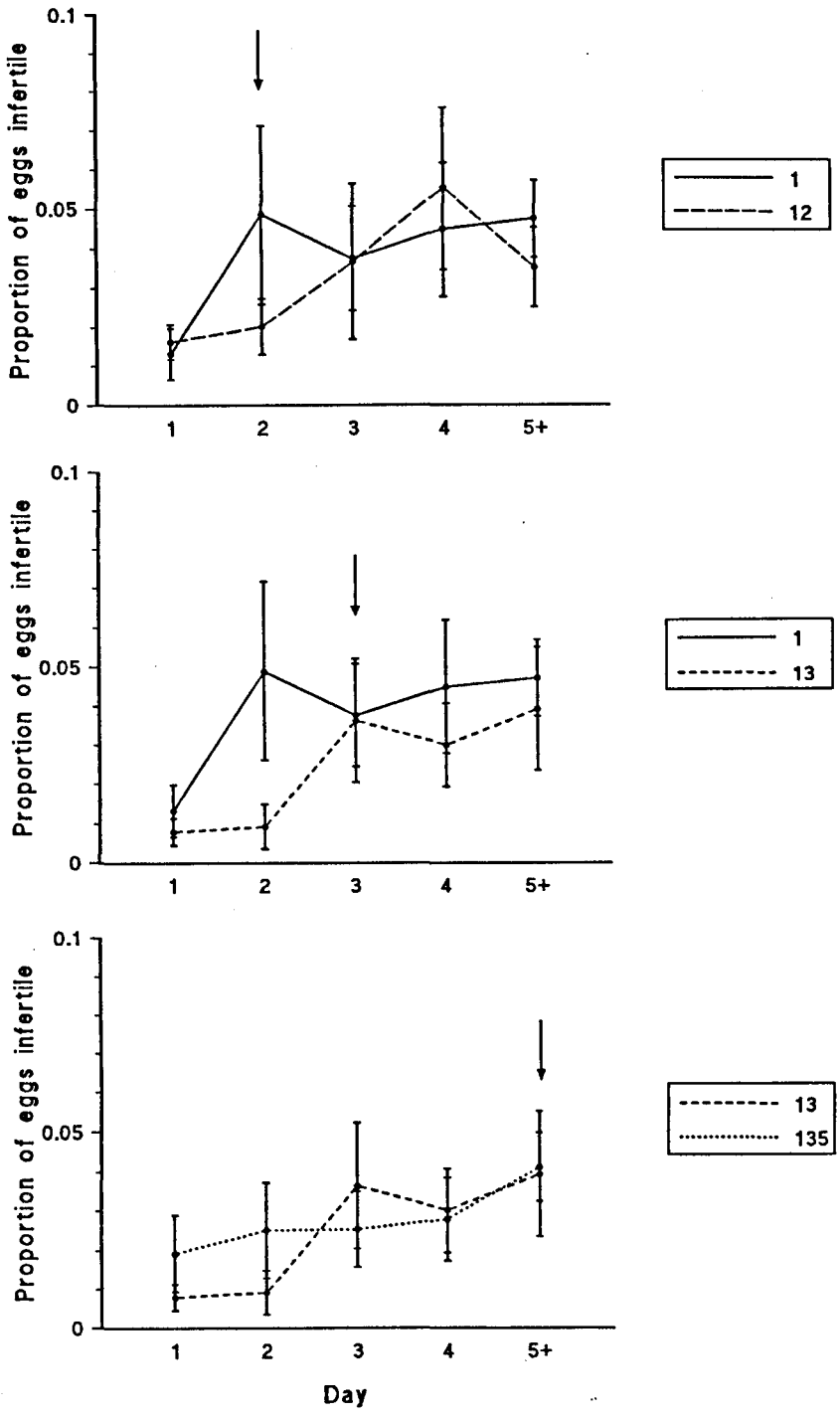

Fig. 6.11 The **proportion of eggs which were infertile,** oviposited on successive days by **moderately-resourced females** with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other.

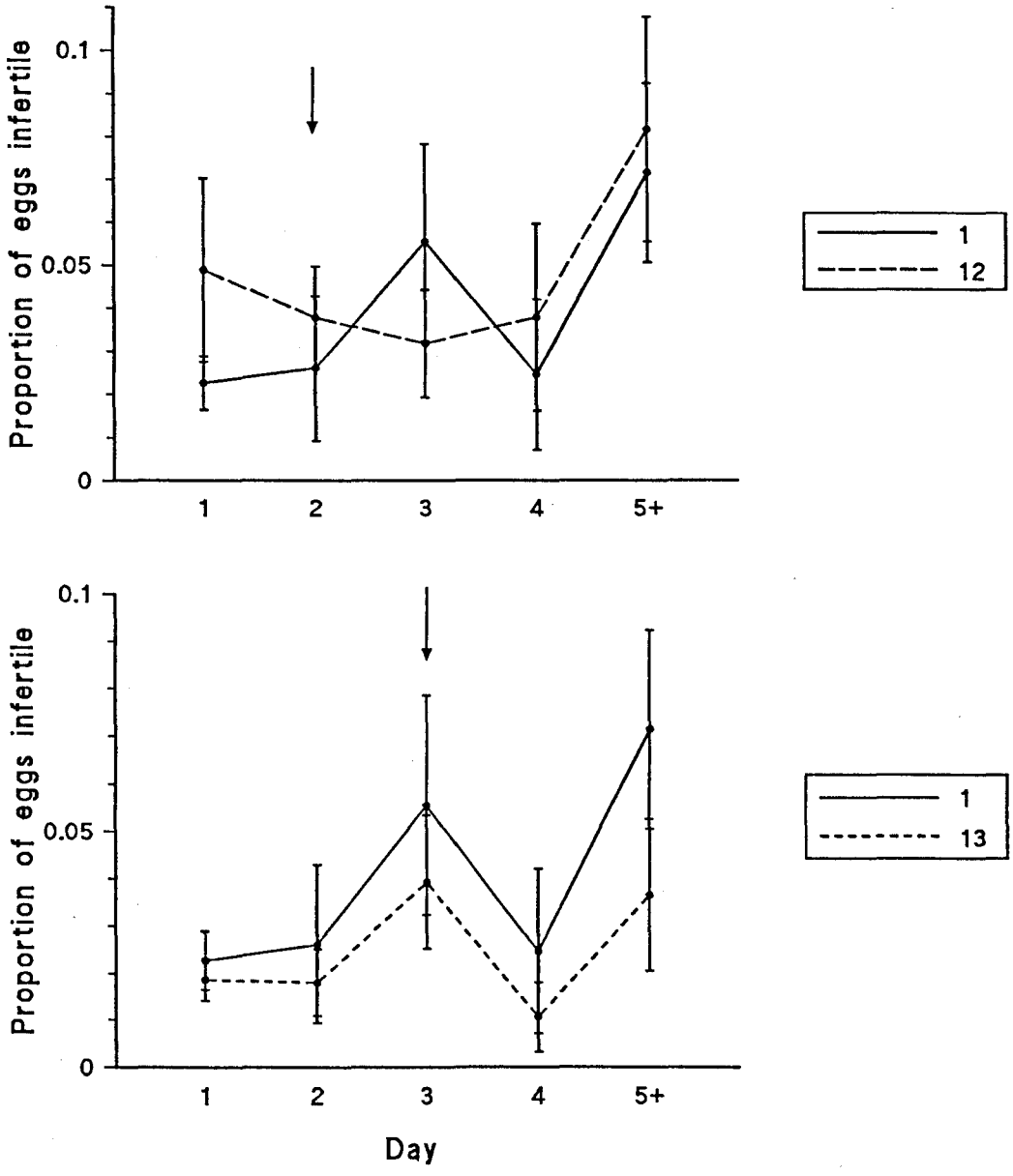

Fig. 6.12 The **proportion of eggs which were infertile,** oviposited on successive days by **poorly-resourced females** with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other.

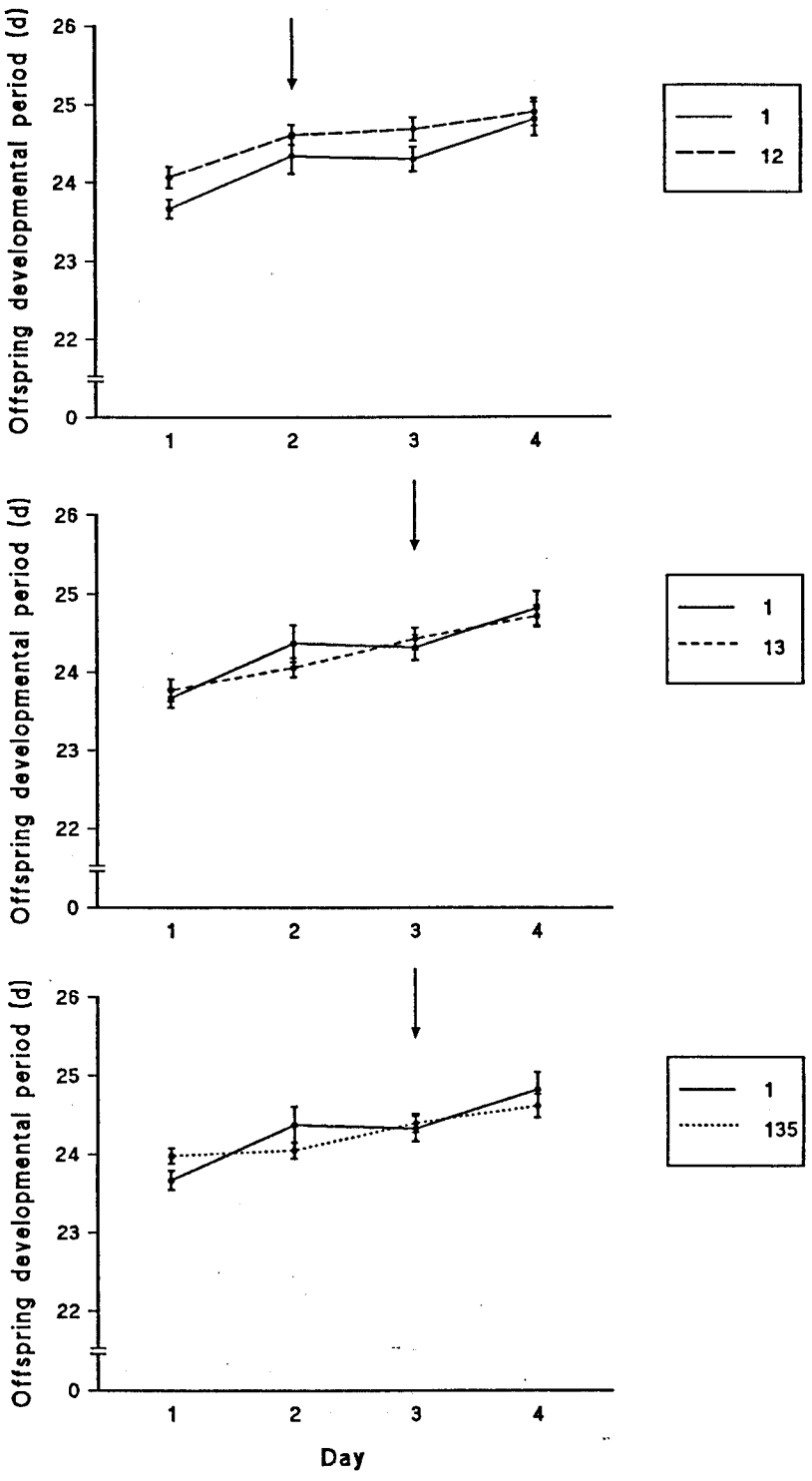

Fig. 6.13 The **mean developmental period of the offspring** from eggs oviposited on successive days by **well-resourced females** with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other.

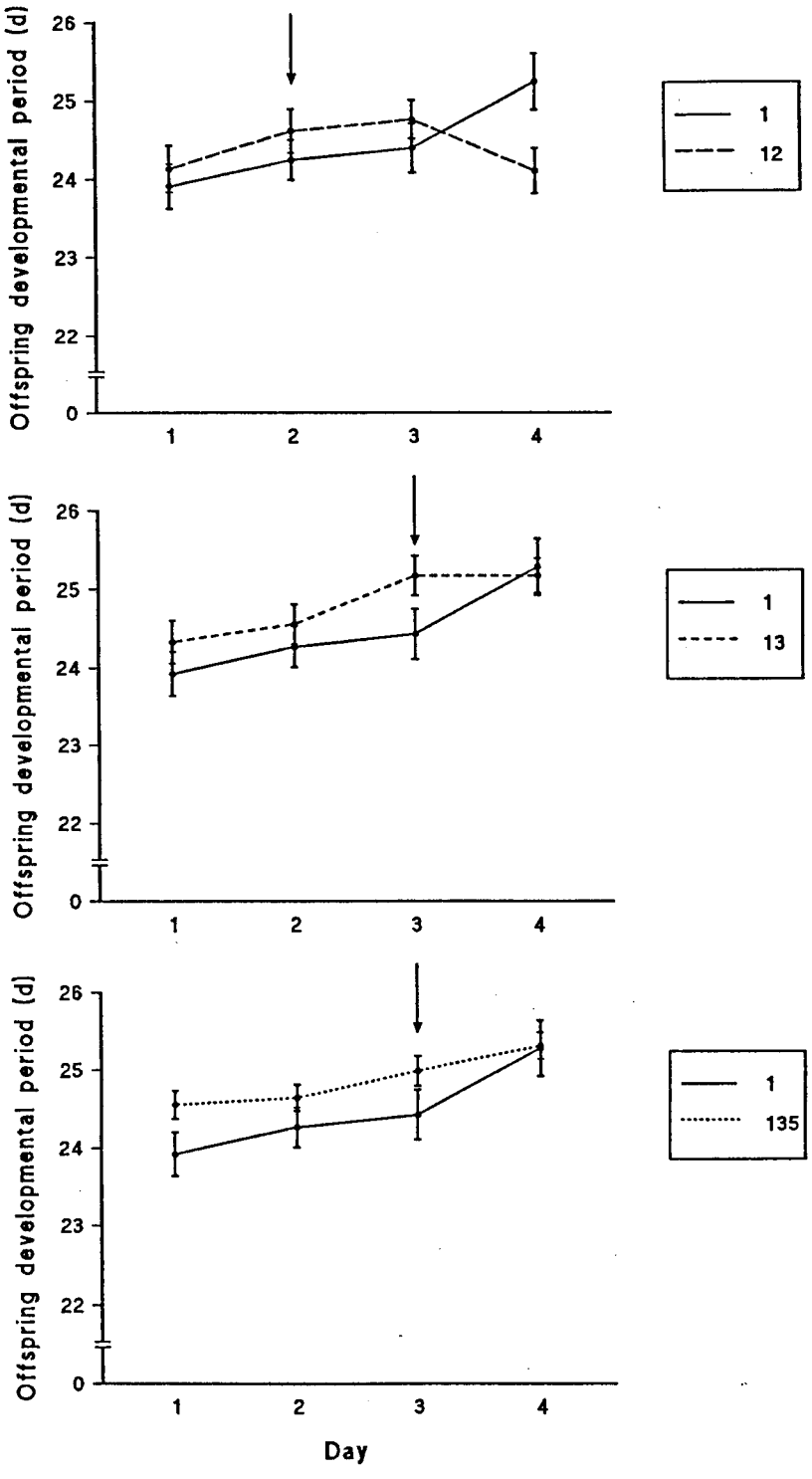

Fig. 6.14 The **mean developmental period of the offspring** from eggs oviposited on successive days by **moderately-resourced females** with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other.

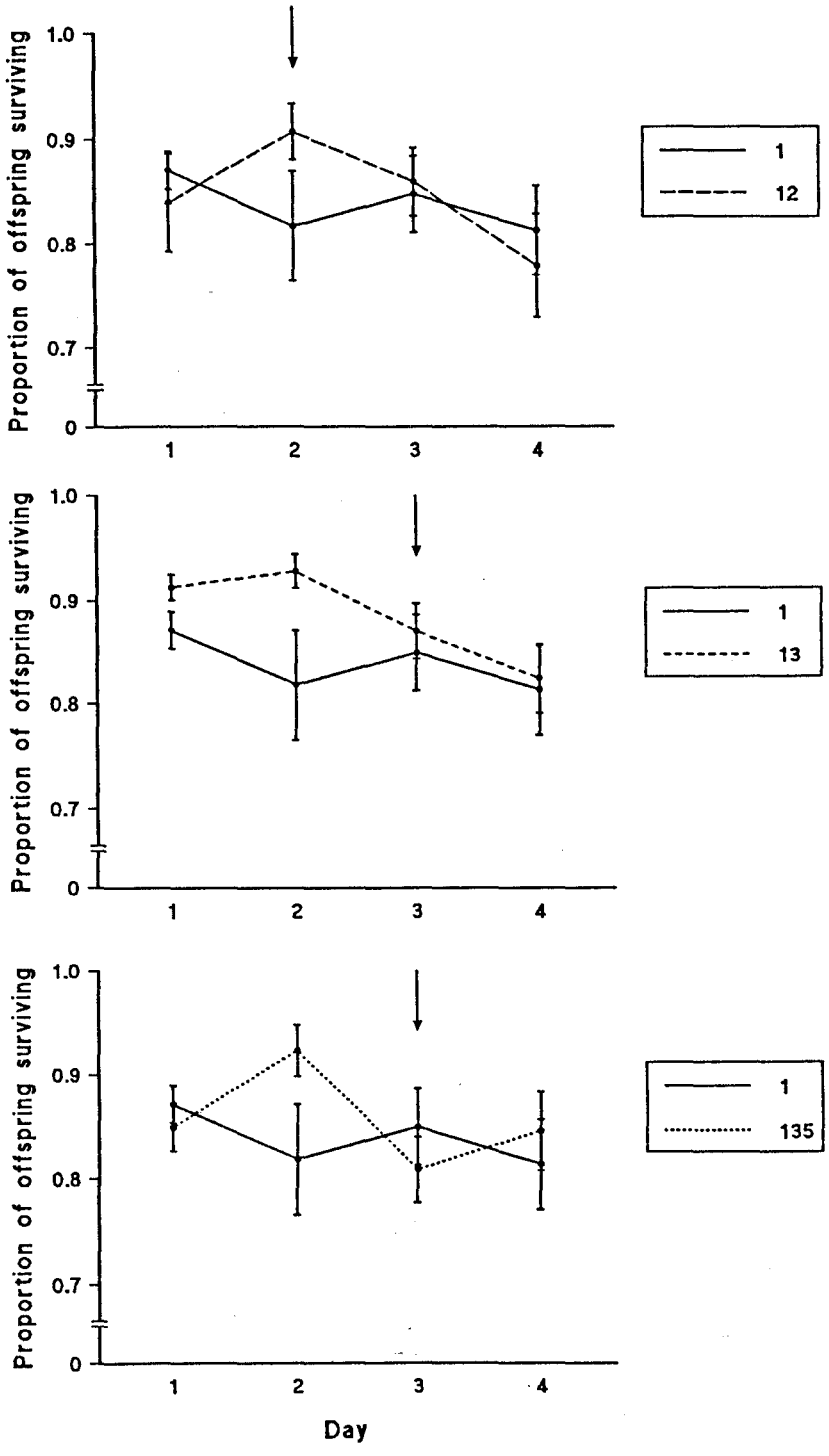

Fig. 6.15 The proportion of fertile eggs from which adults emerged, oviposited on successive days by well-resourced females with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other.

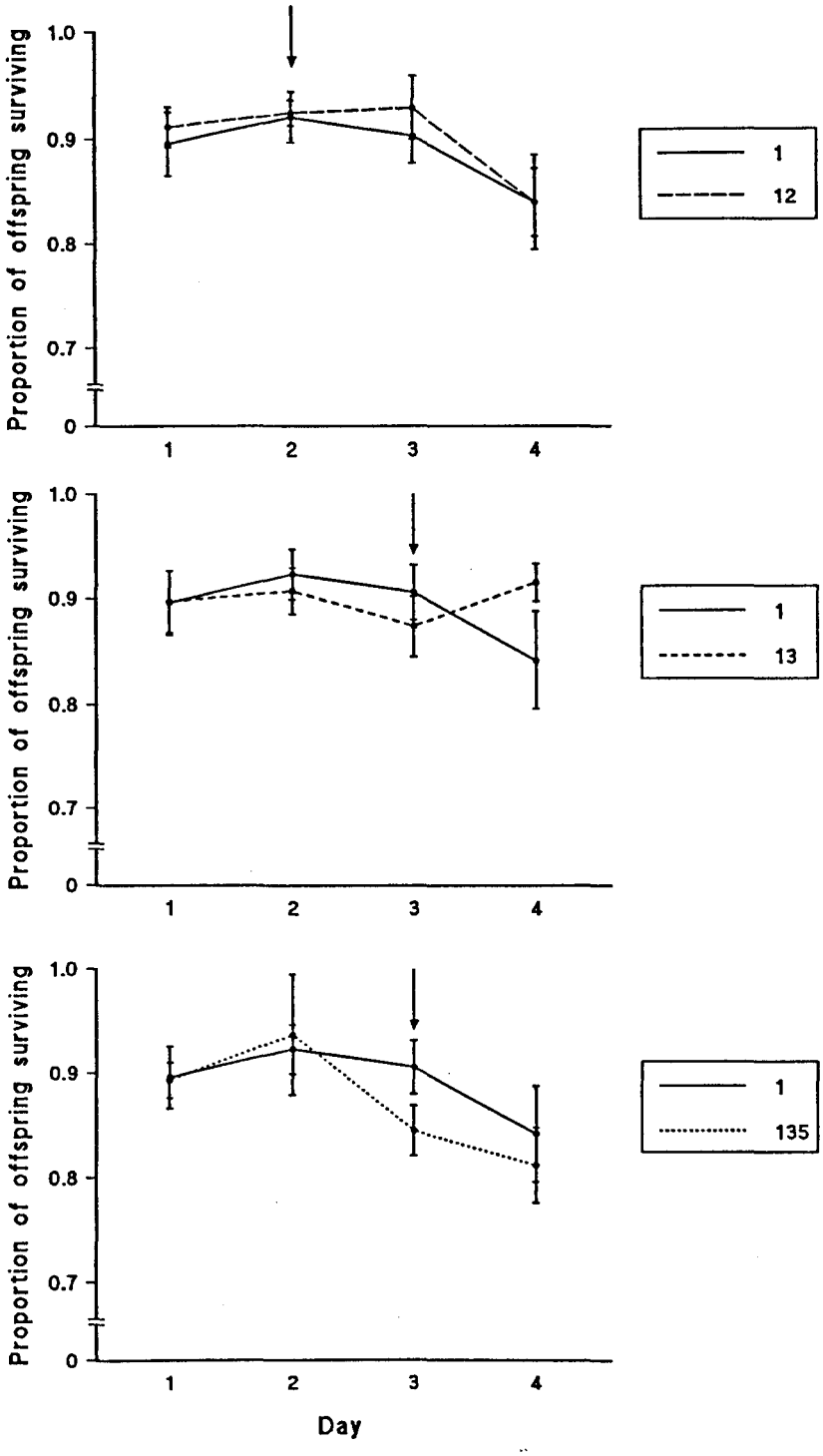

Fig. 6.16 The proportion of fertile eggs from which adults emerged, oviposited on successive days by moderately-resourced females with differing mating schedules (see 6.2.2 for an explanation of the experimental design and treatment abbreviations). Arrows indicate the 24 h period following remating in one of the groups but not the other.

Table 6.6 Two way analyses of variance showing the effects of females' remating treatment and nutritional status on their lifetime fecundity (see 6.2.2): a) Treatments (1, 12, 13) vs. Nutritional status (well resourced, moderately resourced, poorly resourced); b) Treatments (1, 12, 13, 135) vs. Nutritional status (well resourced, moderately resourced). Two analyses were necessary because the poorly resourced group lacked treatment 135.

a)

	d.f.	MS	F	p
Treatment	3	2203.4	7.27	< 0.001
Nutritional status	1	3854.7	12.72	< 0.001
Treatment x Nutritional Status	3	23.9	0.08	> 0.05
Residual	172	303.1		

b)

	d.f.	MS	F	p
Treatment	2	2687.1	9.61	< 0.001
Nutritional Status	1	2354.2	8.42	<0.01
Treatment x Nutritional Status	2	27.5	0.10	> 0.05
Residual	113	279.7		

in the culture boxes (pers. obs.). This suggests that rupturing is not an artefact of the unusual experimental conditions and is likely to be an important cost of copulation in their natural environment. Attempts to avoid the damaging effects of multiple copulations may be the cause of increased 'egg dumping' by small females; Wilson (1989) concluded that the greater amount of oviposition on unsuitable sites by small females deprived of suitable sites was related to the smaller volume of their reproductive tract. If the reproductive tracts ruptured because they were distended by spermatophores, there may be selection for females with smaller reproductive tracts to reduce this distension by dumping eggs. However, the rupturing may be unrelated to the distension of the reproductive tract with spermatophores. It may be due to mechanical damage during copulation. However, this cannot be assessed from these data because the frequency of copulation in each treatment was not measured.

In addition to this extreme cost of repeated copulation, there may be significant courtship costs. The courtship of female *Callosobruchus maculatus* includes the following potentially costly processes: production of attractant pheromones (Rup and Sharma, 1978; Qi and Burkholder, 1982), a courtship ritual (Rup, 1986) and, finally, kicking and dislodging rejected males (Rup, 1986).

6.4.2. Benefits of remating

The fecundity of females was temporarily increased during the 24 h following remating. It is implausible that these increases in daily fecundity on the day following the second day mating in 12 females and the third day mating in 13 and 135 females were a consequence of whether or not females chose to mate on the second day. The hypothesis that these increases were caused by remating is confirmed by the similar increase following the fifth day mating in moderately-resourced 135 females which was not seen in 13 females; yet these females were randomly allocated between groups. In contrast to fecundity, other life-history traits were unnaffected.

i. Benefit from the ejaculate itself.

1) Nutritional role for the ejaculate. There was no reduction in the remating benefit of well-resourced females compared with the remating benefit of moderately and poorly resourced females. Consequently, a nutritional benefit is not proved. However, the effects of remating by females of differing nutritional status are consistent with a nutrient role for the ejaculate. The similar increase in the lifetime fecundity of both well-resourced and moderately-resourced females which mate multiply would be expected if these females were receiving similar nutritional benefits. The lack of an increase in remated poorly-resourced females could be because they are limited by resources which are not contained in the ejaculate. Perhaps the strongest evidence supporting a nutritional role for the ejaculate is that, in spite of an increase in lifetime fecundity, there is no compensatory fall in the values of other measured fitness components. However, if resources were diverted to lifetime fecundity from a range of other traits, the small drops in the values of each of these traits could not be detected without much larger sample sizes. The slight decline in lifetime fecundity of females receiving smaller ejaculates from recently mated males is again consistent with a nutritional role but little weight can be attached to this given the many alternative explanations (see Chapter 5).

2) Compensation for a low quality first ejaculate. The results from Chapter 5 show that females receiving ejaculates with a low fertility lay very few eggs. A remating benefit was revealed even when analysis excluded such females. Consequently, the increase in mean daily fecundity following remating cannot be due to the compensation by some females for infertile first ejaculates. Second, completely infertile males are rare (4 of the 263 copulations between virgin pairs in this experiment resulted in no viable offspring on the first day of oviposition). Females were therefore unlikely to encounter infertile males twice in this study and the third mating benefit would not be expected if remating benefit were due to infertile ejaculate replacement. Compensation for a completely infertile ejaculate can be rejected as the sole cause of a remating benefit.

It is possible that differences in the quality of ejaculates may affect female reproductive success even if all ejaculates were fertile. If there were some mechanism, either female choice

or sperm competition, by which the better quality ejaculates were more likely to be used by a female to fertilise her eggs, remating females would be expected to have a greater lifetime fecundity than once-mated females because they would sample a number of ejaculates and use the best. However, such a mechanism has not been demonstrated and, in any case, would be unlikely to account for such a large effect on the female's fecundity.

A more plausible hypothesis is that remating benefits females by replacing ejaculates of declining quality. This is consistent with the increases in the lifetime fecundities of females remated more than once. However, it does not explain the increase in fecundity with no compensatory decrease in other traits (but see above). Wilson (1989) showed that *C. maculatus* eggs are fertilised shortly before they are oviposited. The suppression of oviposition experiment (section 3.2.4) showed that females using ejaculates inseminated 4 d previously fertilise similar numbers of eggs to females with more recent ejaculates, they continue to fertilise eggs for a further 7 d. This makes a sperm deterioration hypothesis unlikely.

3) Flushing out an infected first ejaculate. Remating appears not to benefit the female by flushing out an infected ejaculate. One would expect an infected ejaculate to affect female longevity or offspring fitness. These traits are unaffected by remating. Furthermore, if there were a lag phase before the pathogen, *e.g.* a bacteria or a virus, had multiplied in number sufficiently to harm the female, an immediate benefit of remating would not be expected. Although, if the female were able to detect the pathogens at low levels, she might divert resources to combat them immediately. More detailed predictions of the effect of pathogen removal on the timing of fitness benefits would depend both on the population dynamics of the pathogen and the relationship between pathogen population size and damage to the female.

ii. Indirect benefit from mating

These experiments made no attempt to investigate the remating benefits to females in terms of reduced male harassment; apart from when copulation took place, these benefits were observed in the absence of males. These benefits could be investigated by comparing the

oviposition of females kept with males to which they had been mated and similar females kept with similar males to which they had not been mated. If males did not harass females to which they had been mated, the first group of females should oviposit at a greater rate. Intuitively, this hypothesis seems unlikely; it would require individual recognition which, as far as I am aware, has not been demonstrated in insects.

6.4.3. Females may not benefit from remating.

i. Females may be forced to mate.

The frequent kicking behaviour of unreceptive females suggests that they can repel unwanted males to a certain extent. If females were unable to repel males, kicking behaviour would be pointless. However, the rupturing of repeatedly mated females suggests that males may be able to force females to mate under certain conditions. For instance, if kicking were energetically costly, females might not be able to sustain this behaviour for long enough to repel all males. Moreover, if there were many males and few beans, females might be unable to escape.

ii. Selection for high mating frequency.

A high mating frequency might occur in females due to the strong selection for this trait in males (Halliday and Arnold, 1987; Arnold and Halliday, 1988). While this hypothesis might explain the frequent mounting behaviour of females and their occasional antennation of males, I am sceptical that it explains remating in this case given the differing male and female behaviours. Males obtain copulations by pursuing and antennating females, whereas females encourage mating by producing pheromones and staying still. There is considerable doubt over the theoretical basis of Halliday and Arnold's hypothesis. Cheng and Siegel (1990) summarise the fairly damning evidence against it: first, they cite Sherman and Westneat's (1988) finding that, in vertebrates, there is no correlation between the variance in mating frequency of males and females; second, they summarise the evidence that in domestic

chickens, there are no genetic or phenotypic correlations between the mating frequencies of males and females and, in particular, that selection for high male mating frequency has no genetic effect on female reproductive traits and that many increased responses of females are caused by the increased activities of males. Furthermore , to support their argument, they reinterpret some of the data originally cited by Halliday and Arnold (1987).

iii. Manipulation by males

The short term increases in daily fecundity following remating are very much what one would expect if males were manipulating the egg maturation or oviposition rates of females. There was no evidence in Chapter 5 that ejaculate depletion affected oviposition rates but males may well continue to produce the 'active factors' in sufficient quantities even when they produced ejaculates containing fewer sperm. Many studies have investigated the effects of injecting accessory gland extracts into the female body cavity (Pickford *et al.*, 1969; Leahy and Lowe, 1967) It is argued that these fluids have a manipulative role if the female responds to their injection by increasing her oviposition rate. This argument is specious; if females benefit from a nutritional ejaculate they would be expected to increase their oviposition rates in response to increased haemolymph concentrations of substances which signalled its presence. The concentration of accessory gland secretions in a female's haemolymph might be a reliable way of determining ejaculate value; whereas the size of an ejaculate would be unreliable if males could control ejaculate size independently of nutrient content.

6.4.3. Distinguishing between hypotheses

The hypotheses discussed above are not mutually exclusive and so none can be rejected with complete confidence. The evidence presented here supports most strongly either a nutritional or a manipulative role for the ejaculate. In my opinion, it is impossible to distinguish between these two roles. Two techniques are often purported to distinguish between nutritional and manipulative effects of the ejaculate: radiolabelling of compounds within the ejaculate and

manipulating feeding regimes. These techniques do not allow the two roles of the ejaculate to be distinguished.

First, radiolabelling of compounds within the male ejaculate may show that male-derived nutrients are incorporated into oocytes. However, this, in itself, does not show that such incorporation has any influence on a female's reproductive success. In fact if suitable compounds were diffusing into the haemolymph, it would be surprising if they were not incorporated. This is what one would expect; nutritionally important compounds are present in the ejaculate as an energy source for the sperm (*e.g.* glutamate in *Drosophila nigomelanica* Chen, 1984) and these would diffuse into the female's haemolymph.

Second, it is argued that if females with access to food do not benefit from remating and starved females do, this indicates a nutritional role. This argument is invalid if remating stimulates feeding. It would also be invalid if feeding has any non-nutritional role in increasing the costs of remating. For example, in *C. maculatus*, where feeding is usually manipulated by providing cotton wool soaked with a nutrient solution, feeding females may suffer greater costs from unwanted copulation attempts than non-feeding females. Thus, any benefits of remating are less likely to be observed.

From the point of view of measuring the cost of reproduction it is critically important whether or not females gain a nutritional benefit from the ejaculate; this would affect the elevation of the trade-off curve. If the nutritional benefit satisfied the requirements of what had been the limiting resource and consequently made another resource limiting, it would alter the trade-off slope.

7. General discussion

The aim of this study was to describe the costs of reproduction in *Callosobruchus maculatus*. In this chapter, these costs are summarised and their implications are discussed in general terms.

7.1. The costs of reproduction

The resources available to adult *Callosobruchus maculatus* are fixed at emergence because they do not feed as adults. Consequently, increased allocation of resources to one trait must reduce the amount available for allocation to others and trade-offs are expected in this species. In this study, a trade-off in females between lifetime fecundity and adult longevity was demonstrated by manipulating the availability of oviposition sites and hence manipulating lifetime fecundity. It was shown that females denied oviposition sites early in their lives were able to oviposit after females able to oviposit throughout their lives had died. Thus, the trade-off reflected the cost of reproduction; current reproduction had a negative effect on future reproduction. It was argued that the trade-off was a consequence of substantial resource investment in eggs; the dry weight of the mean lifetime fecundity of 91 eggs represents approximately 27% of the female's dry weight at emergence. Analysis of the way in which resources were allocated between reproductive and somatic processes showed that females exhausted their resources at the time of death; there was no evidence that one resource in particular was limiting. It would not be surprising if females organised their resources in such a way that these resources were exhausted simultaneously. However, the possibility that the simultaneous exhaustion of resources was coincidental cannot be ruled out. A second cost of reproduction demonstrated in this study was the risk of the female reproductive tract being ruptured by repeated copulation.

Costs of reproduction were demonstrated in males by manipulating female availability and hence the opportunities for copulation. Males suffer both short term and long term costs of reproduction. In the short term, ejaculate supplies are diminished following copulation and there is an interval following copulation in which these supplies are replenished. During this

interval, copulations are likely to result in lower paternity due to reduced effectiveness in sperm competition. The ejaculate will also be of lower value to the female in terms of its fertility and possibly its nutritional value. The long term costs of frequent copulation for males are reduced fertility later in life and reduced longevity. This reduced longevity represents reduced future reproductive value; males which have not previously mated are still completely fertile for several days after the death of frequently copulating males.

There are likely to be equivalent short term costs of reproduction in females but these are less accessible to experimental manipulation. Female *C. maculatus* emerge with approximately 8 mature eggs at emergence and mature additional eggs at a rate of up to 15 eggs per day, depending on the availability of oviposition sites (Wilson and Hill, 1989). The rate of egg maturation is perhaps analagous to the rate of ejaculate replenishment, the size of eggs analagous to the size of ejaculates. Thus, rapid rates of oviposition could perhaps constrain subsequent oviposition due to a temporary lack of mature eggs.

7.2. Generality of the measured costs of reproduction

There are three reasons why these costs might differ between populations. First, females might differ in their investment in each egg. Second, they might differ in rates of non-reproductive resource allocation. Third, they might be physiologically adapted to producing a different number of eggs during their lives. These are briefly discussed below.

The size of eggs laid is affected by factors such as maternal age at the time of oviposition (Ovenden, 1991), maternal body weight (Ovenden, 1991) and may be affected by the number of eggs laid, although the expected trade-off between the number of eggs laid and the size of each of them has not been demonstrated by genetic correlations in this species (Ovenden, 1991). If egg size were important in determining offspring fitness (Capinera, 1977) one would expect females to control egg size appropriately for the range of environments they were likely to encounter. Thus, one would expect the trade-off to be stable between populations unless they differed in optimal egg size.

Factors which might affect optimal egg-size between populations would include differences in: 1) larval competition strategy; 2) host quality and 3) the expected intensity of

larval competition. All of these would affect the fitness consequences to the larvae of differing egg size. If a population had a higher optimal egg size one would expect a steeper trade-off.

The rate at which females use their resources for non-reproductive processes may be affected by female body size or temperature and has been shown to be affected by population density (Wightman, 1978). As well as the scale of metabolic investment, populations may differ in the scale of other non-reproductive processes, such as investment in flight muscles in dispersing/non-dispersing morphs (Nwanze et al., 1986) or investment in oviposition site assessment (Messina and Mitchell, 1989). If females used resources more quickly for non-reproductive processes, the trade-off curve would again be steeper.

One expects populations to be physiologically adapted to the environment in which they have evolved. If two populations were adapted to laying different numbers of eggs during their lifetimes one might expect them to respond differently to manipulations of lifetime fecundity; the efficiency with which they produced a given number of eggs during their life would perhaps depend on the difference between this number and their optimal lifetime fecundity. However, the experimental manipulations of oviposition site availability in this study were carried out using a population of beetles that were adapted to the culturing regime. Within this culturing regime, the quality of oviposition sites is variable. Given this exposure to variation in oviposition site availability, females should have been selected to respond appropriately. Again, the similarity of the trade-offs measured in different populations of *C. maculatus* (see Chapter 3) supports the suggestion that different populations behave similarly to oviposition site manipulations.

7.3. Implications of the costs of reproduction

The cost of egg production and oviposition have profound consequences for how a female should allocate her resources during her lifetime; optimality models of oviposition behaviour for this species in relation to the cost of egg production, time constraints and host availability constraints have been discussed extensively by Wilson (1989a). Here, the extent to which the demonstrated costs of copulation might affect the optimal copulation behaviour of males and females is discussed.

7.3.1. Changing costs and benefits of copulation with age

The probability of copulations taking place as an individual ages depend on the costs and benefits both to that individual and to its potential mate. The following section discusses what this and other studies have shown about the costs and benefits of copulation for an individual, both as they age and as their potential mate ages. A full treatment of the problem would require a game theory approach (e.g. Maynard-Smith, 1982). However, additional measurements are needed before a game theory model of optimal copulation strategies may be constructed. These additional measurements are discussed. Finally, the variables which males and females might be expected to assess before copulating, and which could be manipulated to provide experimental confirmation of the importance of the different costs are discussed.

i The costs and benefits of copulation to the female

The costs of copulation for a female are related to her remaining reproductive potential; if she is unlikely to lay many more eggs: a) in terms of remaining reproductive value, she has little to lose by copulating; b) her reproductive tract will be less distended and less likely to rupture; c) her oviposition rate will be lower (pers. obs.; Begon and Parker, 1986) and this will reduce any fitness cost of time spent copulating. The benefits of a second copulation to a female are expected to become greater as she ages: the value of a nutritional contribution from the ejaculate is increased relative to her diminished nutritional status and, as the possibility that she is running out of fertile sperm increases, so does the value of sperm contained in a second ejaculate. Therefore, one would expect females to become more willing to remate as they increase in age.

Increasing male age had little effect on the fecundity of females if these males had not previously mated (results from Chapter 5). However, previous male mating history will be of critical importance in determining female costs and benefits from remating. First, whether females remated because of a nutritional benefit of the ejaculate or because they were in danger of exhausting their sperm supplies, the benefits of remating would be reduced if the male had recently mated because both ejaculate size (and probably sperm content) and the

number of sperm inseminated would be reduced. Second, the number of previous matings would affect the risk of infection with sexually transmitted pathogens. One would expect females to be more reluctant to mate with recently mated males.

ii. The costs and benefits of copulation to the male

It was argued in Chapter 5 that the costs of copulation for a male are related to the size of his ejaculate. First, because his supply of ejaculate is limited, the more ejaculate a male inseminates now, the less he has available to inseminate future females. Second, because the ejaculate represents a substantial allocation of resources (4% of the male's fresh weight at emergence) it reduces his expected longevity and prospects of subsequent copulation opportunities. These relationships have not been described in detail in this study. A formal model of male mating costs would need to include the effects of male age and mating history (both in terms of timing and ejaculate investment) on rates of ejaculate replenishment, competitive ability and courtship effectiveness.

The problem which will be addressed here is that of how much ejaculate a male should invest. Initially, it is assumed that males are selected to maximise their reproductive benefit per unit of ejaculate invested. The reproductive benefit from a copulation depends both on female quality and the amount of ejaculate invested in this female. The problem is most easily approached using marginal value theorem (e.g. Charnov, 1976). First, consider the case if all females encountered were identical. Marginal value theorem suggests that natural selection would favour males who invested less ejaculate than that required to maximise the female's fecundity. Furthermore, as the reserves invested before finding the next mate increased, males should inseminate the female with increasing amounts of ejaculate (Parker and Courtney, 1984). The costs of finding the next mate would be influenced by female density within the environment and the number of competing males.

The reproductive benefit from a copulation depends on female quality in addition to the amount of ejaculate investment. Factors which would affect female quality are: a) the probability that the female has already mated or will remate later and hence that the male will not fertilise all her remaining eggs; b) her remaining reproductive potential, a function of her

age and previous oviposition and c) the speed with which she will oviposit following mating. If female quality differed between environments but was constant within an environment, males in poorer environments should invest greater amounts of ejaculate per copulation. If however, female quality differed within an environment, males should invest less ejaculate in copulations with low quality females (Krebs, 1978). This is because males should invest ejaculate so that their rate of gain in terms of reproductive benefit equals the average rate of gain for the environment as a whole.

The above arguments considered how males should maximise their reproductive benefit per unit of ejaculate invested. However, the experiments of Chapter 5 demonstrated short-term limitations in ejaculate availability. Consider the case if males were able to replenish their ejaculate supply fully between being presented with one or more females. If a male were presented with a single female he should invest the optimal amount of ejaculate as suggested above. If he were unable to supply this quantity of ejaculate, he should invest all he had. If a male were presented with two identical females, he should invest the optimal amount in each of them. If he were unable to manage this, he should invest half of his available ejaculate in each of them. Any other division of ejaculate would reduce his combined reproductive benefit.

When males have the opportunity to copulate, they are not assured of another mating opportunity. If the probability of a second mating opportunity approaches zero males should behave as if they only have one opportunity and invest optimally in the first female. As the probability of a second mating increases, males should reduce their investment in the first female and reserve ejaculate for the second. However, a male can never be certain of a further mating opportunity and should always invest more in one copulation than he reserves for the next. This was observed in Chapter 5, males split their ejaculates disproportionately between subsequent females (this was also shown by Eady, 1992).

The problem is more complicated than this, however. It was suggested in Chapter 5 that copulation was energetically costly to the male and reduced his ability to court females and hence increased his between mating interval. This should increase his investment in a female, simply due to marginal value theorem. It would also reduce his probability of meeting

another female while his ejaculate supply was diminished. This would also increase his optimal ejaculate investment in the current female.

A further problem is that males have a finite lifetime supply of ejaculate as a consequence of resource limitation. During each copulation opportunity, the male must decide how much of the remaining lifetime ejaculate supply he should invest now and how much he should save for future copulations. The optimal strategy depends on: a) how much of his reserve of resources will have been used before the next copulation opportunity and hence how much ejaculate he will have available in the future; b) his probability of survival to the next mating opportunity and c) his expected reproductive return from future copulations. Thus, the optimal allocation strategy changes with male age. A similar problem concerning the optimal allocation strategies between reproduction and storage for perennial plants has been approached using dynamic programming (Iwasa and Cohen, 1989). However, in perennial plants, as for most organisms, it is appropriate to assume that the probability of survival is independant of age. In *C. maculatus*, as a consequence of resource limitation, lifespan is more or less pre-determined and furthermore is reduced by investment in the ejaculate. It would be singularly inappropriate to assume that it was independant of age.

7.3.2. Behavioural assessment of copulation value

Because male experience is critical in determining female benefits from remating, females should assess this. If courtship were sufficiently taxing for a male, it might be possible for a female to assess whether or not a male had recently courted. That males do not court the same female continuously but in bouts (pers. obs.) suggests that they are unable to sustain courtship for prolonged periods. One might expect the length of time for which males are able to sustain courtship to be reduced if they had recently mated.

The previous section suggests that males might be more reluctant to mate if they were investing a large spermatophore. These males are likely to assess both the female and her environment before copulating. First, the male should assess her remaining reproductive potential. It is tempting to suggest that male antennation of the female's elytra and abdomen

during courtship could provide information about her egg or resource content in the same way that antennal perception of resonance by female parasitoids may give an indication of host quality (Klomp and Teerink, 1962; Strand and Vinson, 1983). Second, one might predict that males should be sensitive to the presence of other males. Increased male: female ratios would increase the interval between consecutive mating opportunities and would therefore reduce the probability that males encountered another female before his ejaculate were replenished. Conversely, they might reduce willingness to mate because of an increased possibility of sperm competition.

Both the behavioural mechanisms and the optimality problems outlined above are accessible to an experimentation. Female quality is readily measured and manipulated, the rate at which males encounter females may be controlled and the outcome of sperm competition could be assessed using colour morphs. Rates of ejaculate replacement and the extent to which they are affected by male age and previous mating experience may be measured; Eady (1992) has developed methods for assessing ejaculate sperm content. Finally, the trade-off between ejaculate investment and longevity may be measured by experimental manipulations.

Appendix

Standard errors of the mean resource contents of beetles were calculated as follows (C. Cannings, pers. comm.):

We have some X with mean μ and variance σ^2, where X is the resource content of a beetle.

We measure $Y = (X_1 + X_2 + ... + X_p)$, where p is the number of beetles in a sample,

so, Y has mean $p\mu$ and variance $p\sigma^2$ as values of X_i are assumed to be independent.

Now, we estimate μ by $\hat{\mu} = \bar{y}/p = \Sigma y_i/(pn)$, where n is the number of samples.

$E(\hat{\mu}) = E(\Sigma y_i/pn) = E(Y/p) = E(X) = \mu$

$V(\hat{\mu}) = V(\Sigma y_i/pn) = (1/(pn)^2).V(\Sigma y_i) = (1/(pn)^2).n.V(y_i) = (1/(p^2n)).p\sigma^2$

Now, we estimate the variance of Y, which is $p\sigma^2$, by s_Y^2

so, $V(\hat{\mu}) = s_Y^2/(p^2n)$

i.e. $s.e.(\hat{\mu}) = s_Y/(p\sqrt{n})$

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