THE EVOLUTION OF OVIPOSITION BEHAVIOUR IN THE BRUCHID *CALLOSOBRUCHUS MACULATUS*.

by

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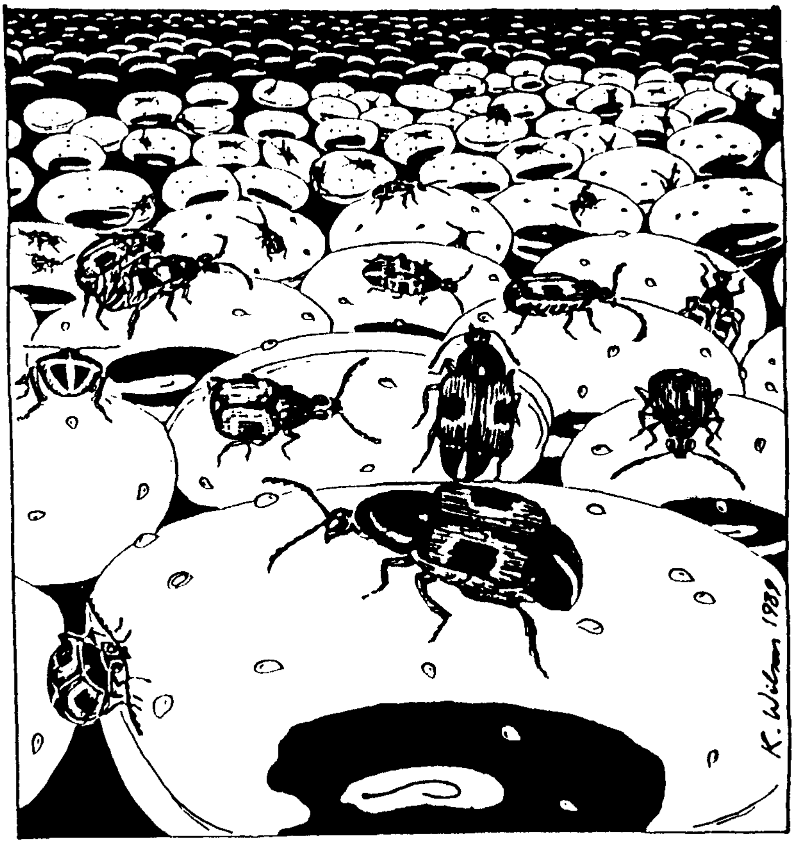

Let him that hath understanding
count the number of the beast.

Revelations of John.

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Ken Wilson, July 1989

ABSTRACT.

Callosobruchus beetles lay their eggs on beans and peas, where the larvae spend all of their pre-reproductive lives. Because survival and fecundity of offspring declines as larval competition increases, the decisions made by an ovipositing female have profound effects on her fitness. Natural selection will therefore favour females that distribute their eggs optimally. The present study uses this assumption to identify the key selection pressures acting on the evolution of clutch size in the bruchid Callosobruchus maculatus.

Chapter 1 provides background information on the study species, discusses the problems elaborated on in following chapters and describes some of the methods used.

In Chapter 2, functional models for oviposition behaviour are described in which assumptions about the major constraints on clutch size vary. It is concluded that whilst some models can be distinguished using qualitative criteria alone, others can only be separated after making quantitative predictions.

Chapter 3 tests some of these quantitative predictions and concludes that time is probably the major constraint on clutch size given that several other females will also lay on the same oviposition sites. However, temporal variation in clutch size, especially with respect to the female's phenotype, suggests that the number of eggs available to females may also constrain clutch size.

Chapter 4 examines the cues and rules used by ovipositing females to discriminate between seeds that differ in egg-load or weight. It concludes that females use the surface area of a seed as a cue to its weight and that a common mechanism may be used to distinguish between seeds that differ in egg-load or weight.

Chapter 5 examines possible physiological constraints on clutch size. The rate of egg maturation and the egg-storing capacity (ESC) of the female may be important constraints when seeds are encountered at high rates.

In Chapter 6, these physiological constraints are incorporated into mechanistic models for clutch size determination. In these models, clutch size is determined by the interaction between external cues, such as those identified in Chapter 4, and external cues, implicated in Chapter 5. Models that include physiological parameters explain significantly more of the variance in clutch size than models that include only external cues. Egg-complement relative to ESC appears to be an important factor determining clutch size in this species.

Chapter 7 discusses some of the general conclusions of the study.

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CHAPTER 1

INTRODUCTION.

Chapter 1. Introduction.

Natural Selection and Optimality Theory.

When Charles Darwin (1859) proposed natural selection as the mechanism adapting animals and plants to their environment, the theory he replaced was that of Devine Creation. Since then, natural selection (or more specifically the adaptionist program) has been criticised by some evolutionary biologists as being a Panglossian paradigm (e.g. Gould & Lewontin 1979; see Mayr 1983). In other words, of interpreting every trait of an organism as an adaptation *ad hoc*. However, as Darwin himself pointed out, we cannot expect animals to be perfectly adapted to every aspect of their environment. Indeed, this was one of his main arguments against the natural theologian view of adaptation: if adaptation was the result of supernatural design, then organisms would surely be perfectly adapted to their environment; God would not create anything less than perfect. In On the Origin of Species, he wrote, "We can plainly see why nature is prodigal in variety, though niggard in innovation. But why this should be a law of nature if each species has been independently created, no man can explain".

Darwin cited several examples of animals that could be viewed as being 'sub-optimal'. These included species of upland geese which, he claimed, rarely if ever saw water, yet had webbed feet. In this example, Darwin was concerned with design constraints associated with the phylogenetic origin of the species (upland geese have webbed feet because their distant ancestors had webbed feet), but other, behavioural and physiological, constraints may also be important. Modern Darwinian theory predicts that traits closely associated with fitness will be optimally designed given these constraints.

An area of behavioural ecology that particularly lends itself to testing optimality theory is that of clutch size evolution.

Clutch Size Evolution.

Many animals lay their eggs in discrete batches, known as clutches, which may vary considerably in size both between and within species. The study of clutch size evolution is arguably one of the oldest areas of behavioural ecology (Godfray 1987). Some 130 years ago, Darwin (1859) noted the immense variation between dipteran species in the number of eggs that they lay, "one fly deposits hundreds of eggs, and another, like the hippobosca, a single one". It was not until David Lack's studies of avian clutch size (1954, 1966) that a theory was proposed to explain variation between individuals of the same species. Lack proposed that females were selected to maximise clutch productivity (see later). The 1960s and 70s were characterised by a flood of studies on clutch size evolution in birds; roughly a third of which supported Lack's new theory (see review by Lessells 1986).

Insect clutch size models.

More recently, attention has shifted away from birds towards insects. This has been for two main reasons. The first is that many of the insects that have been studied are economically important pests or parasitoids and, initially, interest was generated by a desire to gain a better understanding the factors important in determining the size and composition of their clutches (e.g. Waage & Hassell 1982, Waage & Godfray 1985). Armed with this information, biologists hoped to be able to reduce their numbers or use them as biological control agents. The second is that the consequences of oviposition decisions of insects are often more easily measured than those of birds, because they do not live as long and can be reared in the laboratory. This makes them ideal subjects for studying optimality theory.

Functional models of insect oviposition behaviour have tended to concentrate on the optimal number of eggs for a female to lay in each clutch. Whilst this decision is often considered in isolation, the new generation of clutch size models increasingly incorporate additional decisions, such as the sex ratio of the clutch (e.g. Waage & Ng 1984, Waage & Godfray 1985, Godfray 1986b), the size of the eggs (e.g.

Smith & Fretwell 1974, Begon & Parker 1986, Parker & Begon 1986) and the proportion of normal and trophic eggs (Godfray 1987). One prediction common to all of these models is that females should never lay clutches that are larger than the most productive (i.e. that producing most survivors). There is strong empirical support for this prediction. However, virtually no species in which this has been examined actually lays the most productive clutch size, suggesting that there are nearly always additional selection pressures acting on clutch size (see Godfray 1987).

This problem was first encountered by biologists interested in the clutch size decisions of birds (Lack 1954). One suggested solution was that birds laying smaller clutches live longer and so produce more clutches and ultimately more offspring (Williams 1966, Charnov & Krebs 1974). Analogous models have been constructed for insects (Ch.2), and a prediction common to all of these rate-maximising models is that clutch size should decrease as the interval between clutches gets shorter. Qualitative support for this prediction comes from a variety intra- and inter-specific studies of oviposition behaviour in butterflies and parasitic wasps (Godfray 1987 and references therein).

Another prediction common to all insect clutch size models is that the size of the clutch will increase as host quality increases. Because the quality of a host is often determined by its size, this means that large clutches should be laid on large hosts. There is now good evidence from a range of parasitic wasps that clutch size is altered in response to the size of the host (e.g. Klomp & Teerink 1962). However, these observations still only provide qualitative support to a whole series of different models.

The rate of development of clutch size models has far out-paced the rate of appropriate empirical studies. Clearly, it is only by testing specific quantitative predictions that it will be possible to distinguish between the array of different models that abound. Good quantitative support for any of these models is still lacking (Godfray 1987). The present study uses the bruchid Callosobruchus maculatus to test the predictions of some of these models.

The Approach.

There are two types of model used by behavioural ecologists. 'Functional' models describe why an animal behaves the way it does; 'mechanistic' models describe how the animal behaves as it does. While functional models have an obvious bearing on evolutionary questions, mechanistic models may also lend insight into the evolution of oviposition behaviour. The present study examines both types of model.

Functional models.

The starting point in constructing a functional model is to identify appropriate 'constraints' and 'currencies' (Ch.2 and Cheverton *et al*. 1985). Constraints describe the limits of an animal's strategy set (i.e. what it can and cannot do), whilst the currency is the commodity that the animal is selected to maximise (this is ultimately fitness but in the short-term may be oviposition rate, rate of energy intake, etc.). Accurate identification of appropriate constraints and currencies is based on a knowledge of the natural history of the animal (e.g. Kacelnik 1984, Cheverton *et al*. 1985, Smith & Lessells 1985).

For example, because bruchids do not usually feed as adults, females carry the resources for a finite number of eggs (about 80 per female). This suggests that eggs may be limiting and therefore that females may be selected to maximise fitness gain per egg (by laying just a single egg per seed). Alternatively, if the most productive clutch size was, say, 5 eggs/host, and females usually encountered just 1 or 2 hosts during their lifetime, then eggs would be relatively abundant and hosts relatively scarce. Under this scenario, natural selection would favour females that maximised fitness gain per host (by laying the most productive clutch size on each host). After likely constraints and currencies have been identified, they can be incorporated into various functional models and examined.

Functional models developed in the present study (Ch.2) make predictions about how the clutch size of ovipositing females should respond to variables such as the host encounter rate and the current egg-load of hosts. Some of these models can be distinguished using qualitative criteria alone, whilst others require precise quantitative predictions to be tested. If a single functional model was implicated by a variety of quantitative and qualitative tests, then this would strongly suggest that the most important selection pressures acting on Callosobruchus oviposition behaviour had been correctly identified. Failure to implicate a single model, would imply that assumptions about constraints and currencies were incorrect and that new functional models should be formulated.

Mechanistic Models.

Functional models are of general applicability but say nothing about the way in which the optimal solutions are derived in any particular situation. The question of how optimal solutions are achieved can be answered on two levels. The first is on a purely descriptive level, by describing procedures for achieving optimal solutions. These procedures have been variously referred to in the literature as 'decision rules', 'rules of thumb', and 'algorithms'. There are many possible decision rules for any given functional model. Two possible rules for ovipositing insects are, "leave the host after laying n eggs", and "leave the host after t seconds".

The second way to answer the question of how optimal solutions are derived is in terms of the internal and external 'cues' used by the animal in making its decisions. Implicit in any decision rule are assumptions about these cues. For example, the oviposition decision rules described above make assumptions about the forager's ability to count the number of eggs it has laid, and to accurately measure time. Before decision rules can be adequately tested, these assumptions about cues need to be verified from behavioural or physiological observations.

Mechanistic models are tested in a similar way to functional models: the animal's behaviour is compared with a variety of qualitative and quantitative predictions. Implication of a single model would indicate that the mechanisms involved in oviposition behaviour were well understood. Aspects of these mechanisms could then be included in refined functional models as 'rule of thumb' constraints.

The Aims

The specific aims of the present study are as follows:

(1) To review functional models that are relevant to clutch size determination in bruchids and, when necessary, to develop new models based on explicit constraint and currency assumptions (Ch.2).

(2) To distinguish experimentally between the above models and so gain insight into the main selection pressures acting on bruchid oviposition behaviour (Ch.3).

(3) To identify the cues used by females to distinguish between seeds of different quality (size or egg-load) and to compare simple decision rules for discriminating between them (Ch.4).

(4) To identify physiological constraints on clutch size (Ch.5).

(5) To incorporate these behavioural and physiological constraints into a mechanistic model that accurately describes the clutch size decisions made by female *C. maculatus* (Ch.6).

Why use Callosobruchus beetles?

Callosobruchus beetles are ideal animals for studying insect oviposition strategies, for several reasons:

(1) The larvae mature in the seed chosen by their mother; their inability to migrate between seeds means that they are unable to change decisions made by her (Mitchell 1975).

(2) Larval competition within seeds is reflected in increased mortality and reduced fecundity of offspring via reduced emergence weights (see Ch.3); each wrongly placed egg may therefore be costly to the female.

(3) Adult bruchids do not usually feed and so reserves accumulated during larval development are finite and must be directed into egg production and maintenance; each egg therefore represents a major investment by the female and selection should act against females that fail to distribute their eggs optimally.

(4) Unlike parasitic wasps, bruchids are not able to determine the sex ratio of their clutches and do not produce trophic eggs. Therefore, calculation of the optimal clutch size is not complicated by these additional selection pressures.

(5) Some functional and mechanistic models have already been developed for these beetles, but they have not yet been adequately tested (see Mitchell 1975, Smith & Lessells 1985).

(6) The bruchid life-cycle takes just four weeks, therefore the consequences of the female's decisions are quickly determined.

Biology of Callosobruchus maculatus

Life-cycle

The southern cowpea weevil, *Callosobruchus maculatus* (F.) is a bruchid pest of stored pulses and leguminous crops. Females of the species lay their eggs singly onto the testa of host seeds. The larvae burrow into the seed and consume large quantities of the cotyledon before pupating and finally emerging from the seed as a reproductive adult. Adults do not usually feed, although they will drink if offered water or sucrose solution (Howe & Currie 1964).

Three or four days after oviposition (at 30°C), the black head of the first instar larva becomes visible through the translucent egg-shell. A day or so later, the larva burrows into the seed, and the chorion fills with cotyledon, giving the egg an opaque white appearance. The larva continues to feed within the seed during its next three instars before finally pupating under a thin 'window' of testa when it is about 26 days old (Bellows 1982a). Two days later, the mature adult emerges and the cycle is completed. Reproducing adults emerge weighing 2-10 mg wet weight (depending on the larval environment) and usually live for

7-10 days (but virgins may live for up to three times as long; present study). Both sexes will mate within an hour of emergence from the seed, and females start laying eggs soon after this (El-Sawaf 1956, unpubl. data). Adults may mate several times during their lifetime (Bellows 1982a, personal observation).

Temperature and humidity influence life-history parameters such as oviposition rate, development rate, mortality rate (e.g. Schoof 1941, Howe & Currie 1964, Giga & Smith 1983, 1987). The optimal conditions for maintaining C. maculatus appear to be 30°C and 70% r.h. (Bellows 1982a).

Oviposition behaviour.

When a female first encounters a seed, she walks over its surface for about a minute and then, having decided where to lay, she stands more or less still with her antennae pointing posteriorly. She then moves her body slowly back and forth until oviposition begins. After ovipositing, she remains motionless for 10-20s before repeating the process or moving on to a new seed. During oviposition, the female deposits a glue onto the egg which helps to attach it to the seed surface. She also lays down an oviposition deterrence pheromone or 'oviposition marker' (Messina & Renwick 1985a, b; Ch.4).

Stock Cultures.

Geographical origin.

Bruchids are distributed thoughout the tropics and sub-tropics (Southgate 1978, 1979), and there may be marked differences in various aspects of their life-history between geographical strains of the same species (see e.g. Credland *et al*. 1986, Messina & Mitchell in press). For this reason, the present study used a single strain of *C. maculatus*. The strain was collected from Brazil in 1974 (R.H. Smith pers. comm.) and has been maintained at Imperial College at Silwood Park since 1977. Animals derived from these stocks have been cultured at Sheffield University since July 1984. The strain is the same as that used by Bellows (1982a, b).

Culturing conditions.

All stocks were maintained on cowpeas (black-eyed beans) *Vigna unguiculata* in a constant environment room at 30 ± 1 °C with a 16 h light : 8 h dark photoperiod. From July 1984 to March 1986, the humidity was 35 ± 5% r.h., and after this time it was maintained at 70 ± 5% r.h. Unless otherwise stated, all experiments were performed at 70% r.h.

Stock maintenance.

Stock cultures were maintained as follows: approximately 200 adult beetles (0-4 days old) were anaesthetised with carbon dioxide, removed from a stock box set up 4 weeks earlier, and transferred onto approximately 1000 pristine cowpeas. Beetles were allowed to oviposit for up to a week (by which time all were dead) before being sieved off the egg-laden seeds. The seeds were left for a further 3 weeks, by which time adults had been emerging for 0-4 days. These newly emerged beetles were then used to set up the next stock box, and the process repeated.

This cycle takes 4 weeks, so there were four stock boxes, and, because there was no mixing between boxes, each box contained a genetically isolated population (sub-stock). Thus one problem of this culturing method is the possibility of genetic divergence between the four populations. This was minimised by transferring about 200 individuals to each new stock box. Variation within experiments was minimised, as much as possible, by using females from a single sub-stock for the duration of each experiment.

General Methods.

Obtaining virgin beetles.

Virgin females of known maximum age were obtained by isolating individual seeds containing pre-emergence adults in ($8cm^3$) cells of square repli dishes (Gallenkamp Ltd., Loughborough) the requisite time before the start of the experiment. All females that were found alone in a cell were then of known age (within limits) and oviposition experience, and were virgins. Females were generally mated to virgin males of similar age. Age at first mating ($\leq$ 24 h) does not affect the subsequent oviposition rate of the female, or the hatching success or degree of dispersion of her eggs (unpubl. data). Beetles were handled with a fine artists paint brush.

Emergence weight and body size.

Emergence weight (within 12 h) or elytra length was used as measures of body size, as convenient. Emergence weights were measured, to the nearest 0.001 mg, using a Cahn 29 automatic electrobalance, after first anaesthetising animals under carbon dioxide for several minutes. Elytra lengths were measured within 12 h after death, to the nearest 0.025 mm, using a stage microscope and micrometer eye-piece. Emergence weight and elytra length are strongly correlated ($r^2 = 0.87$, $n = 28$, $P < 0.001$).

Statistics and computing.

Statistical tests were performed using MICROTAB (Higgenbotham 1985) or Stats Pack (Rosewell 1984) on a BBC microcomputer, or MINITAB (Minitab Inc. 1985) or SAS (SAS Institute Inc. 1985) on the University of Sheffield IBM 3083 computer. Statistical methods were obtained from Sokal & Rohlf (1981), Snedecor & Cochran (1967) and Siegel (1956).

CHAPTER 2

FUNCTIONAL MODELS OF OVIPOSITION BEHAVIOUR.

Chapter 2. Functional Models of Oviposition Behaviour.

INTRODUCTION.

Numerous functional models have been developed in recent years to account for the variation in clutch size within species. The aim of the present chapter is to review those models that are particularly relevant to clutch size evolution in bruchids, and where necessary to develop new models. In Chapter 3, these models will be tested against the behaviour of real animals; the ultimate aim being to gain insights into the main selection pressures acting on bruchid oviposition.

Lack (1954, 1966) was the first biologist to consider the problem of how many eggs a female should lay in a clutch if she is to maximise her fitness. He suggested that the optimal solution is to lay the number of eggs that maximises clutch productivity (which he defined as the number of birds fledging from a clutch, see Fig. 2.1). For organisms that lay just one clutch, or where maximum offspring <u>per capita</u> fitness coincides with maximum brood productivity (Fig. 2.1(b), Godfray 1987), the optimal clutch size probably will be Lack's solution (i.e. the most productive clutch size). However, these conditions are not usually met, and clutch sizes smaller than that which is most productive are commonly observed in birds and insects (see Lessells 1986

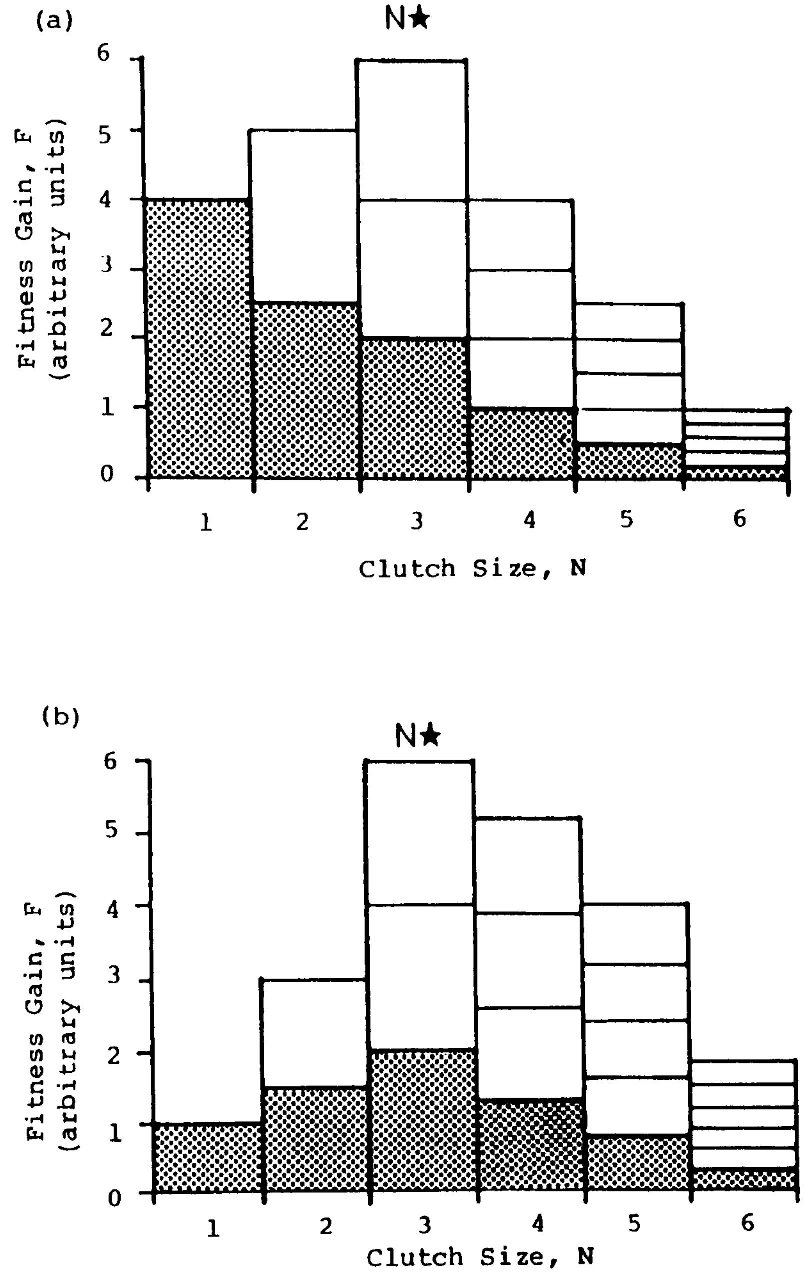


Figure 2.1 Relationship Between Clutch Size and *Per Capita* Fitness (after Godfray 1987).

Shaded histograms represent the fitness of a single offspring as a function of clutch size. Unshaded histograms represent clutch fitness. In (a), the *per capita* fitness curve is monotonically decreasing (as for *C. maculatus*); in (b) the fitness curve is domed and peaks at clutch size three (as for *Zabrotes subfasciatus*, Utida 1967). In both (a) and (b) the most productive clutch size, N*, is three.

and Godfray 1987, respectively, for reviews).

Two explanations for this discrepancy have generally been invoked. The first is that there is a trade-off between current and future reproduction (Williams 1966; Charnov & Krebs 1974), such that females producing clutch sizes below that predicted by Lack live longer and so produce more clutches. The second is that the number of young surviving to breed is an incomplete measure of clutch fitness (eg. Williams 1966, Charnov & Skinner 1984). This latter explanation is particularly likely to be true for insects, where lifetime egg production of offspring may vary several-fold due to differences in their body sizes.

In recent years, the development of optimal foraging models (e.g. Charnov 1976, Parker & Stuart 1976) has renewed interest in the problems of clutch size evolution. Part of the reason for this has been the realisation that offspring production is more closely related to fitness than is feeding: variation in the amount of food eaten is not necessarily translated into variation in fitness, whereas that in offspring production usually is. The new generation of clutch size models are most appropriate for insects, and the relative ease with which insects can be experimentally manipulated means that the models' predictions can often be tested more easily than can similar models for avian clutch size.

Optimal foraging models can be adapted for looking at oviposition strategies in two ways: the first is to consider patches as being composed of a collection of oviposition sites, such as seeds or larvae (e.g. Cook & Hubbard 1977, Hubbard & Cook 1978, Waage 1979); the second is to treat each larva or seed as a separate patch (e.g. Parker & Courtney 1984, Charnov & Skinner 1984, 1985, Iwasa et al. 1984, Skinner 1985, Smith & Lessells 1985). Both types of patch are of finite size and offer diminishing returns to an ovipositing female. This chapter will concentrate on models based on the second of the two patch definitions.

Theoretical Models.

The optimality approach has emphasised the importance of explicitly detailing the assumptions of the models being tested (e.g. Cheverton _et_ _al_. 1985, Stephens & Krebs 1986). There are two main types of assumption: those concerning _constraints_ on the optimal solution (physiological, behavioural and environmental) and those related to the _currency_ being maximised. In the discussion above, the trade-off between current and future reproduction represents a constraint, whilst the number of offspring or the number of grand-offspring resulting from a clutch represent two of the many possible currencies.

The approach taken in the remainder of this chapter is to determine how the predictions of clutch size models vary under differing assumptions. Chapter 3 will ascertain, by experimentation, which of the models the animal's behaviour most resembles, and hence which assumptions are most important. It is stressed that the aim of studies such as this is not to test the paradigm that natural selection works, as has recently been suggested (Lewontin 1983, Pierce & Ollason 1987), but to determine the major evolutionary constraints on behaviour and so gain insights into the main selection pressures acting on the organism (see e.g. Cheverton *et al*. 1985).

A criticism of the optimality approach has been that, when models do not fit, *ad hoc* explanations are invoked (see Stephens & Krebs 1986, for a defence of this technique). It is hoped that by detailing, *a priori*, as many models as possible using realistic assumptions, that this criticism will be inapplicable to the present study.

Currency Assumptions.

The fitness of a reproducing female is ultimately measured by the frequency of her genes in future generations. Lack (1947) used clutch productivity as a proximate measure of fitness, hence the currency he assumed to be maximised was the number of fledglings per clutch. Lack's model often fails because the currency assumptions appear to have been violated.

Currency has two components: the first is an appropriate measure of fitness gain (for clutch size models this means 'total offspring fitness', TOF, see below); and the second is the scale over which this is measured (per host, per minute, per egg, etc., depending on the constraints prevailing). When hosts are limiting, and hence constraining clutch size, the currency being maximised is TOF per host; when time is limiting, TOF per unit time is maximised, etc. Thus, for clutch size models, the currency being maximised is total offspring fitness per unit of limiting resource.

Consider the first component of currency: For many birds, total offspring fitness is likely to correlate reasonably well with fledging success. However, for insects like bruchids that are confined to a single host during their entire growth period, competition between offspring is reflected not only in increased mortality but also in reduced weight at emergence, and hence fecundity (see Smith & Lessells 1985). Emergence weight must therefore be incorporated into any measure of offspring fitness (Charnov & Skinner 1984, 1985, Takagi 1985, Skinner 1985, Smith & Lessells 1985). Clutch productivity corrected for these body size effects will henceforth be referred to as 'total offspring fitness' or TOF.

Total offspring fitness is often approximated by the number of female offspring produced multiplied by their potential fecundity (as estimated by body size). Number of female offspring is used, rather than the total number of offspring, because usually much less is known about the fitness consequences of body size for males than for females. Therefore, whilst this definition of TOF is incomplete (Smith & Lessells 1985), it is generally the best available.

Because of the effects of larval competition, the rate of fitness gain accrued by the ovipositing female changes as clutch size increases. Some authors have called this trajectory of fitness gain the 'larval competition curve' (Smith & Lessells 1985, Credland _et al_. 1986), or the 'number-fitness relationship' (Skinner 1985). For _C.maculatus_ the larval competition curve, based on the production of daughters is convex at all clutch sizes observed (i.e. an Allee effect is not apparent, but see Giga & Smith 1981; Allee _et al_. 1949).

Now consider the second component of currency. If the number of fledglings produced by a female is limited by the number of clutches she can produce in a season rather than the number of fledglings from a single clutch, then time becomes the major constraint on clutch size, and the currency being maximised becomes the number of fledglings per season, rather than per clutch. Hence, currency assumptions are intimately associated with constraint assumptions. By altering assumptions about the major constraints on behaviour (and hence about the currency being maximised) and comparing these results with observed behaviour, information may be gained about the main selection pressures acting on clutch size in this species. Only those constraints that directly affect the currency assumptions (i.e. limiting resources) will be considered in the remainder of this chapter, additional physiological, behavioural and environmental constraints will be discussed in later chapters.

Constraint Assumptions.

The major constraints that have been considered in the formulation of insect clutch size models are hosts, time and eggs. These constraints may be operating singly or in combination and may or may not act independently of each other (Smith & Lessells 1985). For example, time and eggs may each, independently, be limiting, such that in any given situation, the number of eggs available or the amount of time left for oviposition, or both, may affect the optimal clutch size. Alternatively, eggs and time may both be limiting and dependent on each other. This would be the case if reserves (energy, water, nutrients etc.) were limiting and could be directed into either egg production or maintenance: if the female put most of her reserves into egg production, then she would limit the amount that was available for maintenance and consequently her lifespan would decrease (i.e. time would become the major constraint); alternatively, if she put most of her reserves into maintenance, then she would limit the amount that was available for egg production and she would run out of eggs before her death (i.e. eggs would become the major constraint).

In the following section, six basic clutch size models are described. The models differ in the assumptions that they make about which of the constraints listed above contribute most to clutch size decisions. These assumptions are considered singly and in combination, and in one of these models, two constraints are assumed to be dependent on each other. Only those predictions that are considered relevant to bruchid beetles are discussed (most of which are also relevant to gregarious parasitoids).

The six basic models consider the following constraints:

1. Hosts.
2. Time.
3. Eggs.
4. Reserves (eggs & time, dependently).
5. Eggs & time, independently.
6. Eggs & hosts, independently.

Models 1-6 consider the optimal clutch size when a single female is laying and does not revisit hosts. The predictions generated by these models are summarised in Table 2.2. Models 7-12 consider how the optimal solutions change if more than one female lays on each host. Table 2.3 summarises these predictions. In the text, model parameters are represented by the symbols given in bold lettering (summarised in Table 2.1).

Symbol	Explanation
N	Clutch size
N*	Most productive clutch size (Lack's solution)
E	Total number of eggs available (potential fecundity)
T	Total time available for laying (adult lifespan)
To	Time taken to lay each egg
Tt	Time between successive encounters with hosts
@	Total number of clutches
^	Optimal solution (e.g. N^ = optimal clutch size)
-	Value predicted by the Marginal Value Theorem (e.g. N-)
s(N)	Fitness function, relating *per capita* offspring fitness to clutch size
s'(N)	First derivative of above function with respect to N
x	Current egg-load of host
xmax	Predicted maximum egg-load
i	Number of ovipositing females

Table 2.1 Symbols Used in Functional Models.

SINGLE OVIPOSITION MODELS.

Model 1. Hosts Limiting.

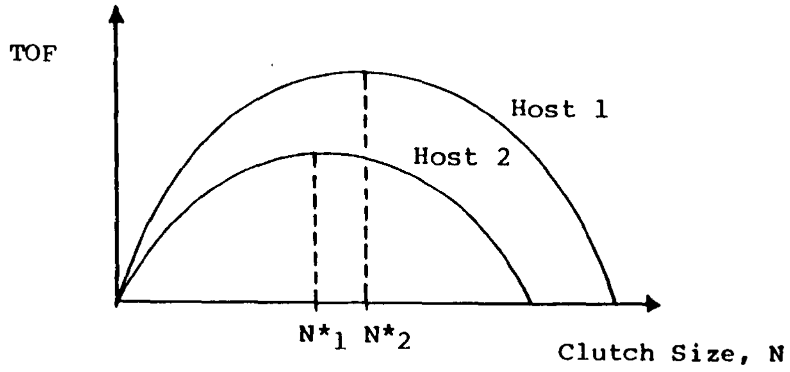
When opportunities to lay clutches are few, but time and eggs available are not limiting, natural selection will favour females that lay clutch sizes that maximise fitness gain per clutch (Charnov & Skinner 1984, Parker & Courtney 1984, Skinner 1985; Fig 2.2(a)). In other words, if the only constraint on oviposition behaviour is the availability of oviposition sites, then the predicted clutch size will coincide with Lack's solution, **N*** (see Appendix 1.1).

Survival from some hosts is higher than from others. This can be for several reasons, but the most obvious one is simply that they are larger and so can support more larvae. Hosts which offer higher than average survival are often said to be of high 'value' (sensu Skinner 1985). The higher the value of a host, the larger its associated **N*** and the greater the number of eggs a female should lay on it. Clutch size is predicted to increase with host value in most of the following models and therefore this result will not usually be made explicit in discussing them.

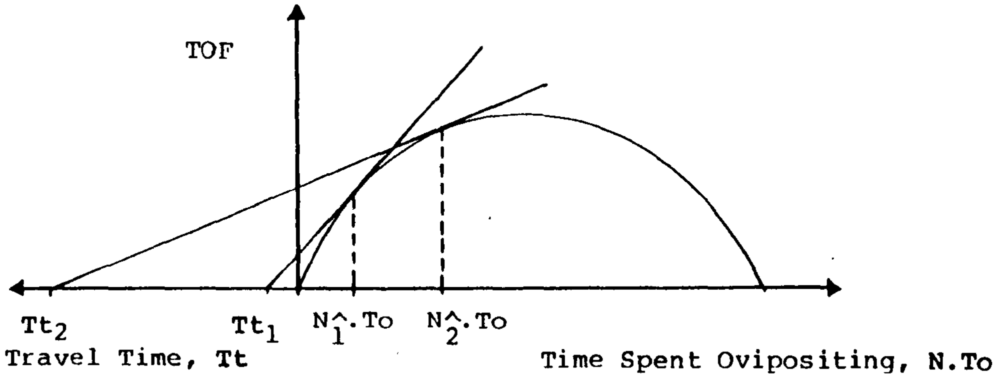
Model 2. Time Limiting.

Laying eggs takes time. If the amount of time taken to

(a) Hosts Limiting



(b) Time Limiting



(c) Reserves Limiting

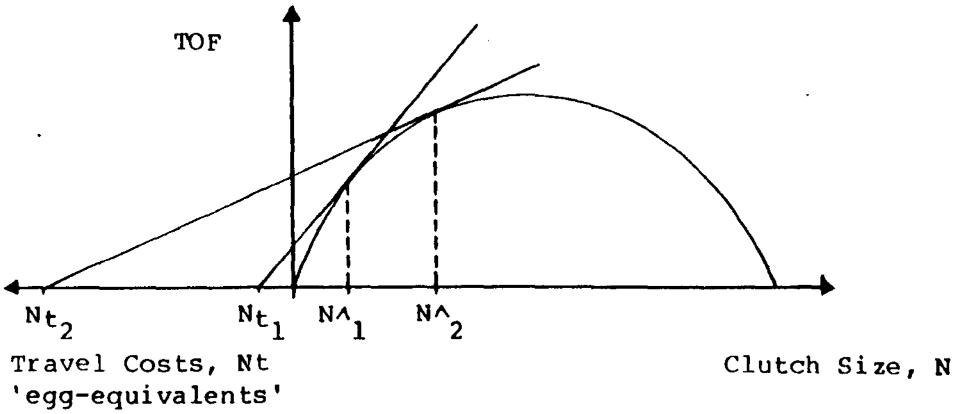


Figure 2.2 Predicted Clutch Sizes When Hosts, Time or Reserves are Limiting.

Each curve represents a fitness gain function relating total offspring fitness (TOF) to oviposition effort. (a) $N^{\wedge}=N^{*}$, the most productive clutch size. Host 2 is of higher value than Host 1 and therefore $N^{*}2>N^{*}1$. (b) Optimal time spent ovipositing ($N^{\wedge}.To$) is found by constructing a tangent from Tt to the fitness gain curve. As Tt increases (from Tt_1 to Tt_2) so the $N^{\wedge}.To$ (and hence clutch size) increases (from $N^{\wedge}_1.To$ to $N^{\wedge}_2.To$). (c) as for (b) except that clutch size replaces time spent ovipositing and 'egg-equivalent travel costs replace travel time.

lay a clutch is dependent on its size, then there is a cost to laying large clutches: as clutch size increases so the amount of time available for searching for new hosts decreases. There is therefore a trade-off between time spent ovipositing on a host (**N.To**, see Table 2.1) and the time spent searching for additional hosts (travel time, **Tt**). This trade-off has been modelled both analytically and graphically using the marginal value theorem (MVT, Charnov 1976; see Fig. 2.2(b) and Appendix 1.2). In these models it is not total offspring fitness (TOF) per host that is maximised, but TOF per unit of time. These models predict that the optimum clutch size (**N**$^{\wedge}$) will be lower than **N*** when **Tt** is short (relative to **To**) and will approach **N*** as **Tt** approaches infinity (Iwasa *et al* 1984, Parker & Courtney 1984, Charnov & Skinner 1984, 1985, Skinner 1985). Kacelnik (1984) and Houston (1987) discuss some of the problems encountered when the rate of fitness gain is not a smooth curve (as assumed by the MVT) but accrues in discrete units, as when eggs are laid. These problems are relatively minor and have generally been ignored in the present study (but see Model 9).

It should be noted that in reviewing previous models of oviposition behaviour, no distinction has been made between models that refer to 'time constraints' and those that refer to 'mortality-risks'. This is because instantaneous mortality rate is equal to the reciprocal of longevity, and therefore models in which mortality-risk is age-independent produce exactly the same predictions as time lmiting models.

Model 3. Eggs Limiting.

When eggs are limiting but time and oviposition sites are plentiful, the optimal solution is to maximise egg fitness (or TOF per egg). For *C. maculatus*, and many other granivores, *per capita* offspring fitness decreases monotonically with increasing clutch size. Hence, $N^{\wedge} = 1$ for accepted hosts (Fig. 2.1(a)). In this context, host value is reflected in the probability of host acceptance rather than in clutch size *per se*. When *per capita* fitness peaks at a clutch size greater than one (as it does for the bruchid *Zabrotes subfasciatus*; Utida 1967), $N^{\wedge}$ is greater than unity and may coincide with N^* (Godfray 1987; Fig. 2.1(b)). In all future discussions of egg fitness, a monotonically decreasing *per capita* fitness curve is assumed.

Model 4. Reserves Limiting (Eggs and Time, Dependently).

Life-history theory predicts that there will be a trade-off between the amount of resources directed into reproduction and the amount directed to maintenance; such that the more eggs a female lays, the greater the reduction in her lifespan (Williams 1966). This sort of trade-off is likely to be particularly important to animals like *C.*

maculatus that do not feed as adults and so for whom resources are limited to the amount accumulated during larval development. The 'cost of reproduction' (Williams 1966) can be modelled using the marginal value theorem (MVT) by specifying a conversion rate between eggs and time (Smith & Lessells 1985).

This model is exactly analogous to the time-limiting model (above), except that the limiting resource has changed from time to egg-equivalents. The predictions of the two models are qualitatively the same (Table 2.2, Fig. 2.2(c)). However, because the cost of oviposition relative to travelling is greater when measured in eggs than in time, observable changes in $\hat{N}$ will only be induced through large changes in the travel times.

Model 5. Eggs and Time Limiting, Independently.

When eggs and time are independently limiting, a reduction in the number of eggs laid does not result in a reduction in lifespan. Likewise, a reduction in the amount of resources directed into maintenance does not necessarily result in increased egg production. Iwasa *et al*. (1984), Parker & Courtney (1984), Waage & Godfray (1985) and Mangel (1989) all produced models that apparently determined the effect of limiting time and eggs on the optimal clutch size. However, all of these studies assumed that **To** (oviposition time) was approximately equal to zero for all clutch sizes.

PREDICTION	MODEL (limiting resource)					
	1. (Hosts)	2. (Time)	3. (Eggs)	4. (Reserves)	5. (Eggs & Time)	6. (Eggs & Hosts)
$\hat{N}$ =	N^*	$\leq N^*$	1	$\leq N^*$	$\leq N^*$	$\leq N^*$
Effect on $\hat{N}$ of increasing:						
Tt	0	+	0	+	+	+
To	0	-	0	-	-	-
Host value	+	+	+[a]	+	+	+
E	0	0	0	+[b]	+	+
T	0	0	0	-[b]	-	-

Table 2.2 Qualitative Predictions of Clutch Size Models: Single Oviposition.

$\hat{N}$ = optimal clutch size; N^* = most productive clutch size; Tt = travel time (time between hosts); To = oviposition time (time taken to lay each egg); Host value is defined in the text; E = number of eggs available; T = time available for laying (adult lifespan).

0 = no effect; + = $\hat{N}$ increases; - = $\hat{N}$ decreases; (a) Host value is here reflected in increased probability of acceptance rather than increased clutch size *per se*; (b) This is the effect of altering the conversion rate between eggs and time. For references see text.

All predictions assume monotonically decreasing offspring *per capita* fitness function.

For explanation of symbols see Tables 2.1 & 2.2. Linear and exponential refer to fitness functions (see text). (a) and (b) refer to model-types (see text).

In other words, the only time constraint they considered was the amount of time available for travelling between oviposition sites (or the risk of mortality between oviposition sites). They neglected the time expended in laying each clutch, which may be considerable for some species. If there are no time-costs associated with large clutches, then the female should lay the clutch size that maximises productivity per host. Therefore, these models are most useful in determining the optimal solution when the major constraints are eggs and hosts rather than eggs and time (see model 6).

When To is not negligible and clutch size is limited by both eggs and time independently, the optimal clutch size depends critically on the ratio T/E (total time available for laying / total number of eggs available; unpublished, Appendix 1.3). Two threshold values of T/E can be recognised: an upper threshold, $(T/E)_u$, above which, the major limiting resource is eggs (because the amount of time needed to lay all of the eggs is less than that available); and a lower threshold, $(T/E)_l$, below which, the major constraint is time (because there is not enough time to lay all of the eggs). Between these two threshold values, the optimal clutch size is that which most efficiently utilises all of the eggs and all of the time available (Appendix 1.3).

The threshold values are given by:

$$(T/E)_u = Tt + To \qquad [2.1]$$

and $$(T/E)_l = Tt + To/N^{\sim} \qquad [2.2]$$

Optimal clutch sizes are as follows:

when $T/E \leq (T/E)_l$ $\quad N^{\wedge} = N^{\sim}$ [2.3]

when $(T/E)_l < T/E < (T/E)_u$ $\quad N^{\wedge} = Tt / (T/E - To)$ [2.4]

when $T/E \geq (T/E)_u$ $\quad N^{\wedge} = 1$ [2.5]

where $N^{\sim}$ is the clutch size predicted by the marginal value theorem (see Model 2).

In other words, above and below these threshold values, the optimal clutch size is independent of **T/E**, but between them, when eggs and time are simultaneously limiting, the optimal clutch size is a decreasing, non-linear function of **T/E** (Fig. 2.3(a)). This means that when there is stochasticity in the value of **T**, the female should reassess the optimal clutch size after each clutch has been laid. If **T(t)** represents the amount of time left at time t and **E(t)** the number of eggs remaining at time t, then $N^{\wedge}(t)$ will be a function of **[T/E](t)** and may result in the optimal clutch size decreasing over time (see Iwasa et al. 1984, Parker & Courtney 1984, Begon & Parker 1986, Mangel 1987, 1989, and model 6 for similar conclusions)

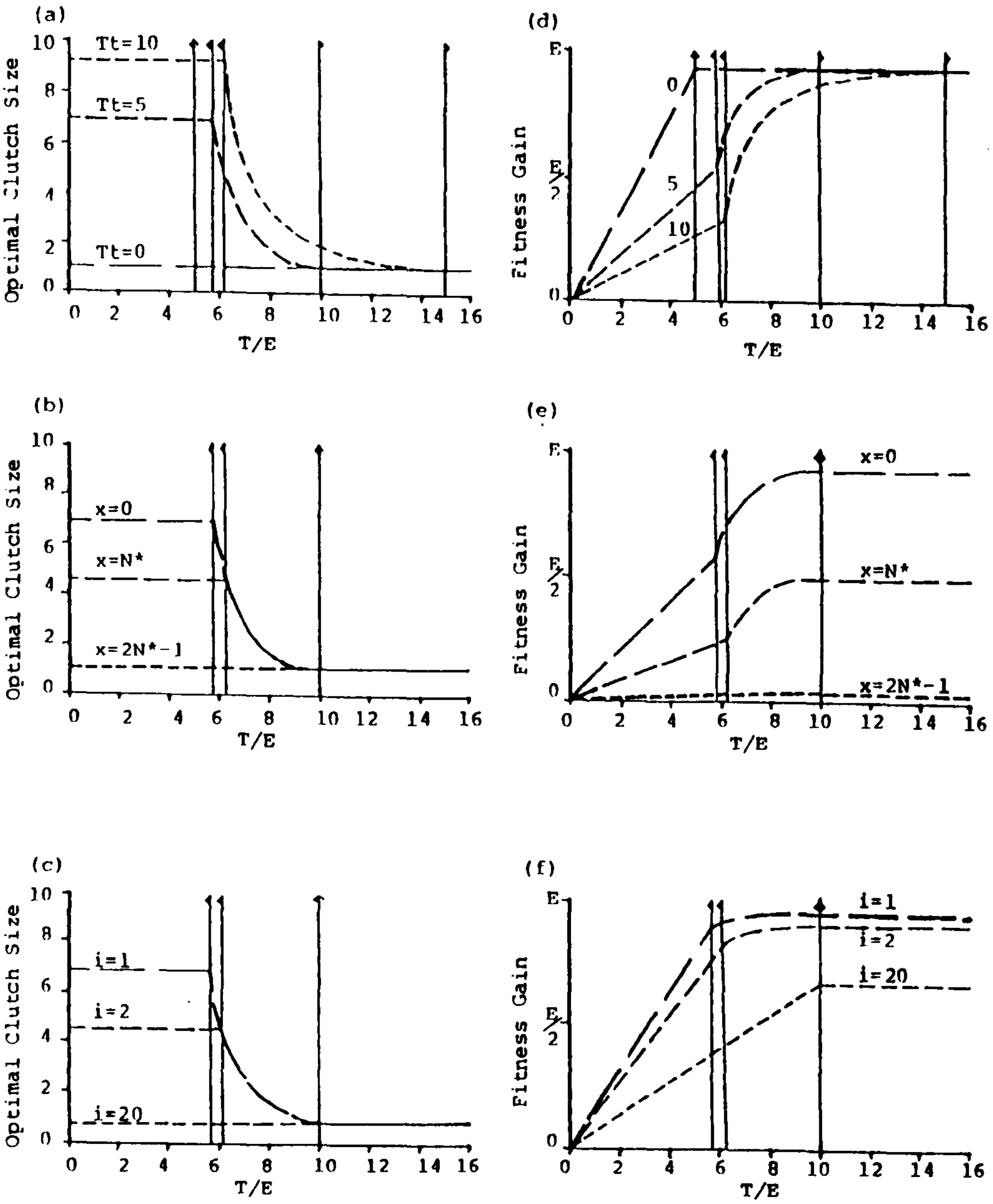


Figure 2.3 Effect of Travel Time, Current Egg-Load, and Number of Ovipositing Females on Optimal Clutch Sizes and Fitness Gain When Eggs & Time Are Simultaneously Limiting (Models 5 & 11).

Figures (a), (b) and (c) illustrate the effect of travel time (Tt), current egg-load (x), and number of ovipositing females (i), respectively, on the predicted optimal clutch sizes. Figures (d), (e) and (f) illustrate the corresponding gain curves. Flagged verical lines illustrate upper (▶) and lower (◀) threshold values for T/E. In the illustrated example E=100, Tt=10 (except in (a) & (d)), To=2, N*=15. See Appendix 1.3 and text for details of calculations and model.

6. Eggs and Hosts Limiting, Independently.

The predictions of this model are determined by combining those for the eggs-limiting and hosts-limiting models. The temporal changes in $\hat{N}$ are considered by the models of Iwasa *et al.* (1984), Parker & Courtney (1984) and Mangel (1987, 1989). These models, originally constructed to illustrate the effect of limiting eggs and time simultaneously (see above), predict that $\hat{N}$ will vary between 1 and N^* as the ratio of eggs (**E**) to hosts (**H**) increases. The transition between the two extreme clutch sizes is linear with respect to E/H (see Fig. 2.4(a), Appendix 1.4 and Iwasa *et al.* 1984).

Iwasa *et al.* (1984) and Parker & Courtney (1984) suggested that $\hat{N}$ will decrease as the female ages because, as she ages, her egg reserves decrease and eggs become relatively scarce whilst hosts become relatively abundant (**E/H** decreases). However, as Mangel (1987) pointed out, this is not always the case, the temporal pattern of $\hat{N}$ depends on the interaction between E and H. For example, depriving a female of hosts for some time may increase $\hat{N}$ because the number of hosts the female can visit before her death decreases, whilst the number of eggs available remain constant, resulting in hosts becoming more limiting (i.e. **E/H** increasing).

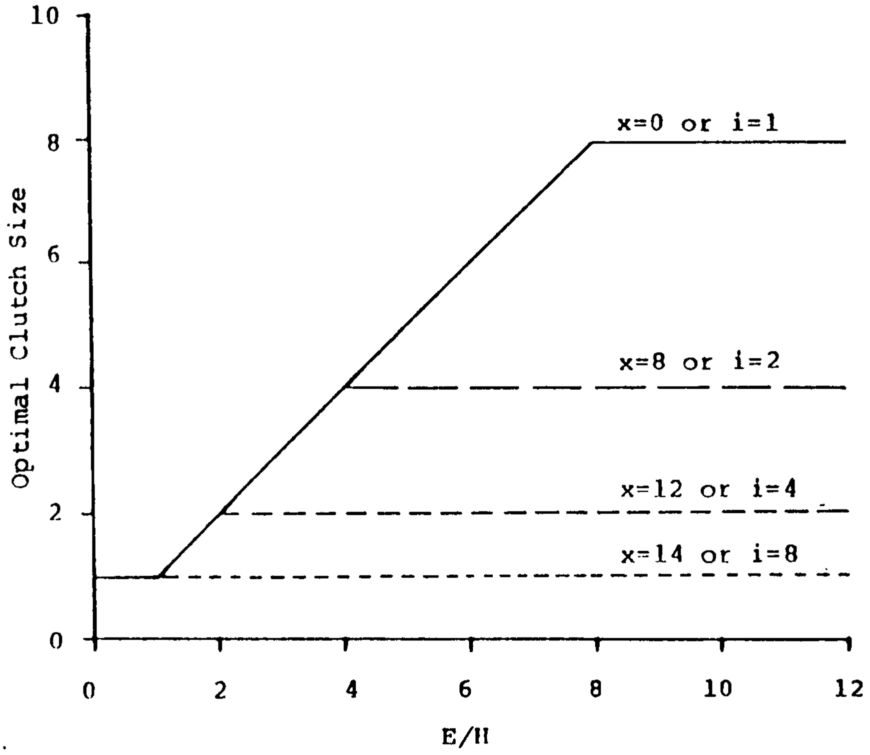


Figure 2.4 Effect of Current Egg-Load (x) and Number of Ovipositing Females (i) on Optimal Clutch Sizes When Eggs and Hosts Are Simultaneously Limiting (Model 12).

In the illustrated example, N*=8. As x and i increase the optimal clutch size for any given value of E/H approaches one. Maximum predicted clutch size is N*. Results are for a linear fitness function (if the fitness function is exponential then N is unaltered by x).

MULTIPLE OVIPOSITION MODELS.

All of the above models predict optimal clutch sizes assuming that each oviposition site is visited just once. However, multiple oviposition (two or more clutches laid on a single host) is common in bruchids, and females are known to respond to small differences in existing egg-loads (Messina & Renwick 1985a, b). Models that ignore multiple oviposition are therefore likely to produce unrealistic predictions.

Two approaches have generally been taken to examine the influence of multiple oviposition on the optimum clutch size. The first, referred to hereafter by the suffix *a*, has been to determine $N^{\wedge}$ for a female that lays last on an egg-laden host (Charnov & Skinner 1985, Skinner 1985, Smith & Lessells 1985; see also Charnov & Skinner 1984, Iwasa *et al*. 1984,); the second, referred to hereafter by the suffix *b*, has been to determine $N^{\wedge}$ for females that 'know' how many females in total will oviposit on each host (Parker & Courtney 1984, Smith & Lessells 1985, Godfray 1987). The former approach uses simple optimisation techniques, whilst the latter requires an evolutionarily stable (ES) clutch size to be determined (Maynard Smith 1974, Parker & Courtney 1984, Smith & Lessells 1985, Godfray 1987).

Most of the multiple oviposition models developed up to now have assumed that the currency being maximised by the female is the rate of fitness gain per unit of time or reserves expended. However, these models can be adapted for other currencies (see below). The predictions generated by these models are summarised in Table 2.3.

Model 7. Hosts Limiting.

The models of Smith & Lessells (1985), which assume a reserves constraint, can be adapted to look at the problem of multiple oviposition when the availability of hosts is the major constraint (C.M. Lessells, unpubl.; see Appendix 1.1).

7a). Consider first, the optimal clutch size ($\hat{N}$) for a female that is the last to lay on a host already bearing **x** eggs (equivalent to model 1a of Smith & Lessells). Total offspring fitness derived from each host is equal to:

$$F(N,x) = N.s(N,x) \qquad [2.6]$$

where **N** equals clutch size, **x** is the current egg-load of the host and **s(N,x)** is the *per capita* fitness of all larvae in the host.

Differentiating with respect to **N** gives:

$$dF/dN = s(N,x) + N.\ s'(N,x) \qquad [2.7]$$

where **s'(N,x)** is the first derivative of **s(N,x)** with respect to **N**.

When seeds are limiting, the optimal clutch size is that at which dF/dN is equal to zero, and therefore $\hat{N}$ can be derived by substituting the fitness function into Eqn. 2.7, and solving for **N**.

PREDICTION	MODEL (limiting resource) 1. (Hosts)	2. (Time)	3. (Eggs)	4. (Reserves)	5. (Eggs & Time)	6. (Eggs & Hosts)
(a) <u>Linear.</u>						
Effect on $\hat{N}$ of increasing x	-	-	0/-	-	-	-
xmax	$2N^*$	$2N^*$	$2N^*$	$2N^*$	$2N^*$	$2N^*$
<u>Exponential.</u>						
Effect on $\hat{N}$ of increasing x	0	0	0	0	0	0
xmax	none	none	none	none	none	none
(b) <u>Linear.</u>						
Effect on $\hat{N}$ of increasing i	-	-	-	-	-	-
xmax	N^*	N^*	N^*	N^*	N^*	N^*
<u>Exponential.</u>						
Effect on $\hat{N}$ of increasing i	-	-	-	-	-	-
xmax	N^*	N^*	N^*	N^*	N^*	N^*

Table 2.3 Qualitative Predictions of Clutch Size Models: Multiple Oviposition.

See legend to Table 2.2.

When the fitness function is linear (Perrins & Moss 1975, Smith & Lessells 1985) and described by:

$$s(N,x) = a - b.(N+x) \qquad [2.8]$$

By inserting Eqn. 2.8 and its derivative with respect to **N** into Eqn. 2.7 and solving for **N**:

$$\hat{N} = a/2b - x/2 \qquad [2.9]$$

In other words, $\hat{N}$ is equal to Lack's solution (a/2b) minus half of the current egg-load. Using this fitness function, there is an upper limit to the total number of eggs expected on a host (2**N***), because above this clutch size fitness equals zero.

A linear fitness function may be biologically unrealistic for bruchids (see later), and so it is necessary to determine $\hat{N}$ when the fitness curve is non-linear. For analyical tractability an exponential fitness function can be used (Bellows 1981, Smith & Lessells 1985, Waage & Godfray 1985):

$$s(N,x) = \exp(-c.(N+x)) \qquad [2.10]$$

and this gives:

$$\hat{N} = 1/c = N^* \qquad [2.11]$$

That is, $\hat{N}$ is independent of the current egg-load, and is affected only by the severity of larval competition. Consequently, with this fitness function, there is no upper limit to the number of eggs expected when all seeds have the same egg-load (but clutch size will be egg-load dependent when egg-loads vary within the environment; see Smith &

Lessells' model 1b). This result is a consequence of the form of the function chosen and may not be generally true.

In their models, Parker & Courtney (1984) used a different non-linear fitness function, such that:

$$s(N,x) = 1 - c.(N+x)^2 \qquad [2.12]$$

Substituting this function and its derivative into Eqn. 2.7 gives:

$$\hat{N} = -2x/3 + (x^2/9 + N^{*2})^{\frac{1}{2}} \qquad [2.13]$$

which indicates that when the fitness function is non-linear (and convex up), the optimal clutch size may be dependent on the number of eggs already on the host (x) and on the severity of larval competition (indicated by N*). An upper limit to the number of eggs on a seed is also predicted using this function (at $3^{\frac{1}{2}}.N^*$). These results underline the warning made by Smith & Lessells about making generalisations from specific functions.

In summary, if the female behaves as if she is the last to lay on a paricular host and the availability of hosts is the major factor limiting clutch size, then she will be sensitive to both the shape of the fitness function and to the current egg-load of the host. These results are consistent with those of Skinner (1985). A further prediction from Skinner's model is that if progeny fitness declines as a function of the age of competing larvae in the host (as is true for some bruchids, Bellows 1982a) then clutch size will decline with time between successive female visits. In all the models that follow, this effect is neglected.

7b). Consider now, the number of eggs a female should lay given that (i-1) other females will also oviposit on the same host (equivalent to model 2 of Smith & Lessells). When the probability of multiple oviposition is high, the ES clutch size will be approximately the same for all females (Parker & Courtney 1984) and can be found by substituting (i-1).N for x in Equations 2.7 & seq. (for derivations see Appendix 1.1.).

When the fitness function is linear (Eqn. 2.8):

$$\hat{N} = a/(2bi) \qquad = N^*/i \qquad [2.14]$$

When the function is exponential (Eqn. 2.10):

$$\hat{N} = 1/ci \qquad = N^*/i \qquad [2.15]$$

And when it is non-linear and convex upwards (Eqn. 2.12):

$$\hat{N} = (1/i)(1/3c^{\frac{1}{2}}) \qquad = N^*/i$$

[2.16]

In other words, the optimal clutch size is dependent on the severity of competition within the host (as indicated by N*), and decreases as the number of females laying eggs (i) increases. For all of the above fitness functions, the total number of eggs per host ($i.\hat{N}$) is equal to Lack's solution (N^*).

Model 8. Time Limiting.

8a). When time is the major constraint, the optimal clutch size declines as the egg-load of the host increases (see Appendix 1.2). This is because the MVT predicts that **N**^ will decrease as host value decreases, and the value of the host is reduced by the current egg-load (see above). The optimal clutch size of the second female will generally be smaller than that of the first female (but may be larger if the second female spends longer searching for hosts; Skinner, 1985). The maximum number of eggs expected on a host (**xmax**) is equal to that at which no adults subsequently emerge (2**N*** for a linear fitness fuction).

8b). When a female is one of a known number of females to lay on a host, her optimal clutch size is dependent on the shape of the fitness function, on the cost of travelling between hosts, and on the number of females ovipositing (Parker & Courtney 1984). The models of Smith & Lessells (1985) can be adapted to demonstrate that as the number of ovipositing females approaches infinity, **xmax** approaches **N*** (see Appendix 1.2).

Model 9. Eggs Limiting.

9a). The optimal solution when the number of eggs available to a female is the only limiting factor can be determined by setting the travel costs to zero in the models of Smith & Lessells (1985). The prediction from all of their models is that N^ equals zero when Tt equals zero. However, this result is a consequence of the functions used, which are all described by smooth curves. As clutch size can only take integer values, the gain curves are best described by step functions. The graphical solution is now $\hat{N} = 1$. Clearly, if time and hosts are not limiting then, in an environment in which all hosts have the same value and egg-load (model 1a of Smith & Lessells), a female that assumes she is the last to lay should always lay one egg on each host, irrespective of its egg-load. However, in an environment with variable egg-loads the optimal probability of laying on a host will be negatively correlated with the number of eggs on its surface. When hosts vary in 'value', the optimal clutch size may be zero for some hosts and they will not be oviposited on. The problem is then analogous to one of optimal diet choice (Iwasa *et al*. 1984).

9b). When i females are laying on the same hosts, the optimal clutch size is again one. This result is more or less independent of the value of i (although if the fitness function meets the abscissa at some point then $\hat{N}$ will equal zero when i is reaches this point).

Model 10. Reserves Limiting (Eggs and Time, Dependently).

10a). The predictions under a reserves constraint are qualitatively similar to those under a time constraint. The optimal clutch size decreases monotonically with increasing egg-load and, for fitness functions that intercept the abscissa at some point, there is a maximum expected egg-load

(Smith & Lessells 1985). As with the time constraint model, large search costs result in an increase in the optimal clutch size.

10b). If the female behaves as if other females will subsequently lay on the same hosts, then the ES clutch size will be a decreasing function of the number of females ovipositing (i) and of the search costs (**Tt**). As **i** and **Tt** increase so the maximum number of eggs on a host approaches N^*.

Model 11. Eggs and Time Limiting, Independently.

11a). The optimal clutch size when eggs and time are indendently limiting is dependent on the ratio **T/E** (see model 5). If all hosts have the same egg-load and value then when the major constraint is the number of eggs available (i.e. $T/E > To + Tt$), the optimal clutch size is unaffected by the current egg-load and is equal to one (see model 9a). When time is the major constraint (i.e. $T/E < To + Tt/\tilde{N}$), the optimal clutch size is $\tilde{N}$, the egg-load dependent marginal value clutch size (which decreases as egg-load increases, see model 8a). At intermediate values of **T/E**, intermediate values of $\hat{N}$ are expected (see model 5), and these are also egg-load dependent (see Fig. 2.3(b)). As egg-load increases, the slope of the transition curve from $\hat{N}=1$ to $\hat{N}=\tilde{N}$ remains constant, but the critical threshold value of **T/E** at which $\hat{N}$ diverges from $\tilde{N}$, increases (see

Appendix 1.3). The situation becomes more complicated if the environment has variable egg-loads: $N^{\wedge}$ is now dependent on the frequency of low egg-load hosts in the environment (analogous to an optimal diet choice model).

11b). The predictions of this model are qualitatively the same as for the model above when all hosts have the same egg-load (see Fig. 2.3(c) and Appendix 1.3).

Model 12. Eggs and Hosts Limiting, Independently.

12a&b). The optimal clutch size when eggs and hosts are limiting depends on the ratio E/H (see model 6 and Appendix 1.4). Increasing the egg-load (x) or the number of females laying (i) reduces each female's N^* (see model 7) and hence lowers the transition value of E/H (Fig. 2.4). When x or i is sufficiently large, $N^{\wedge}$ will equal one and be independent of E/H.

As when time & eggs are limiting, quantitative predictions become more difficult if egg-loads vary within an environment, but one would expect $N^{\wedge}$ to decrease as the current egg-load increased, and that for some hosts the optimal clutch size will equal zero and they will be rejected.

COMPARING THE FUNCTIONAL MODELS.

The predictions of the twelve models are compared qualitatively in Tables 2.2 and 2.3. It is possible to distinguish between some of these models using these qualitative criteria alone, but others can be distinguished only by using precise quantitative predictions (see Ch.3).

Single Oviposition Models.

When the number of hosts is the major constraint, the optimal clutch size is always equal to Lack's solution, **N***, irrespective of the cost of finding each host. This prediction distinguishes the hosts-limiting model from the five other single oviposition (SO) models.

When the number of eggs is the primary limiting factor, $\mathbf{N}^{\wedge}$ is always equal to 1 (or, if host value varies, zero or 1). This prediction distinguishes the eggs-limiting model from the five other SO models.

All four of the remaining SO models predict that $\mathbf{N}^{\wedge}$ will vary between 1 and **N***. Therefore, if the observed clutch size changes as travel time changes, then the hosts-limiting and eggs-limiting models can be rejected. But, if clutch size is constant and equal to **N*** or 1, then this does not necessarily validate models 1 and 3, respectively. This is because ovipositing females may not be sensitive to the prevailing travel times: their response may be evolutionarily fixed (see Ch.3). However, if the observed clutch size is constant and greater than one, then model 3 can be rejected; and if the observed clutch size is less than **N***, then model 1 can be rejected (although the importance of hosts as a limiting resource cannot be discounted). If clutch size varies with respect to **Tt** then models 2, 4, 5 and 6 can only be distinguished by quantitative differences in predictions (see Ch.3).

Multiple Oviposition Models.

The six multiple oviposition (MO) models considered can not be distinguished on the basis of the qualitative predictions listed in Table 2.3. All of these models predict that if females are sensitive to the level of MO indicated by the egg-load of the current host (sub-models a), then clutch size will decrease as the egg-load of the current host increases; and if females respond to an 'anticipated' level of MO (sub-models b), then clutch size will decrease as the anticipated number of ovipositing females increases. It should be noted that this latter response may be evolutionarily fixed, or may be sensitive to the prevailing level of MO (estimated by the number of ovipositing females encountered, for example; see Ch.3).

CHAPTER 3

TESTING THE FUNCTIONAL MODELS.

Chapter 3. Testing the Functional Models.

INTRODUCTION.

A series of functional models for oviposition behaviour were described in Chapter 2. The predictions of several of these models are qualitatively similar and so they can only be compared using quantitative predictions. In order to make such predictions, the relationship between the pay-off (total offspring fitness, TOF) and the limiting resource (hosts, eggs, time, etc.) must be known. This involves determining the shape of the larval competition curve; defining the relationship between clutch size and time spent ovipositing; and specifying a conversion rate between eggs and time (Ch.2). Measuring and interpreting these relationships is not straight-forward and the problems are general ones associated with testing optimality models.

These can be divided into four categories: those associated with measuring fitness consequences (for example, the shape of the larval competition curve); those associated with measuring life-history trade-offs (such as the cost of reproduction); those associated with interpreting qualitative and quantitative data; and those associated with interpreting flexibility in behavioural responses. These difficulties are now discussed with respect to their bearing on the present study.

Measuring Fitness Consequences.

There are two parts to the problem of measuring the fitness consequences of decisions. The first is in deciding on a measurable quantity that approximates fitness. As discussed in Chapter 2, for avaian clutch size models this is often the number of fledged offspring, whereas for insects in which fecundity varies considerably with body size the measured quantity is usually potential number of eggs produced by female offspring (total offspring fitness, TOF). The relationship between TOF and the number of larvae per host is often referred to as the larval competition curve (LCC).

The second part of the problem of measuring the fitness consequences of decisions results from the methods used. There are two methods for determining the relationship between a dependent and independent variable: the observational and the experimental (see Perrins & Moss 1975, Hogstedt 1980, Godfray 1987, Pettifor _et_ _al_. 1988). The observational method is the one most frequently employed, but there are problems associated with it. For example, the LCC may be calculated by determining TOF from naturally-produced egg-loads, but if females lay different-quality eggs in different-sized clutches, or different-sized clutches on different-quality hosts then the the LCC will be incorrectly calculated. Non-random distribution of eggs between clutch sizes will tend to accentuate or diminish the apparent effects of larval competition. This problem is partly overcome by manipulating the independent variable (e.g. egg-loads) experimentally. This is easily achieved for bruchids by removing eggs before they hatch. Both methods were employed in the present study (though only the observational method was used to calculate the LCC; see Materials & Methods and Ch.4).

Measuring Trade-Offs.

The problem of making correct inferences from observational data is a general one, and one which is also encountered when measuring life-history trade-offs, such as the cost of reproduction (Reznick 1985).

If limited reserves can be directed into either reproduction or maintenance, then an increased investment in reproduction must result in a decreased investment in maintenance, and vice-versa. This results in a trade-off known as the cost of reproduction (Williams 1966), which can, like the LCC, be measured in two ways (Reznick 1985).

The first, and most commonly followed method, is to measure the phenotypic correlation between naturally occuring variation in, for example, lifetime egg production and longevity. However, the demonstration of a trade-off using this method relies on there being more variation between individuals in their relative allocations to reproduction and maintenance than in the total amounts of their reserves (van Noordwijk & de Jong 1986). Since many insects, including bruchids, vary several-fold in body size within species, it is unlikely that a trade-off will be detected in the Bruchidae using this method.

The second way of demonstrating the cost of reproduction is to observe the effect of experimentally manipulating some aspect of reproduction, or some environmental variable that affects reproduction. This has the effect of increasing the variance in the allocation to reproduction (and hence maintenance). Egg-production of _C. maculatus_ is reduced in the absence of mates and oviposition sites (Ouedraogo & Huignard 1981, Ch.5 present study), and this provides a possible mechanism by which the amount of resources allocated to reproduction may be manipulated in this species. This mechanism is suitable for measuring the cost of reproduction because it is likely to result in females moving along trade-off curves, rather than between them (see Reznick 1985). Supplementing the diet of adult beetles, on the other hand, would probably result in females moving onto higher trade-off curves and so would be unlikely to demonstrate the existence of a life-history trade-off.

Interpreting Qualitative and Quantitative Data.

As indicated in Chapter 2, some functional models can be distinguished using qualitative criteria alone. However, qualitative similarity between observations and predictions must be interpreted with extreme caution (e.g. Krebs _et al_. 1983, Stephens & Krebs 1986, Ch.2). In the present study, all four of the single oviposition (SO) models that cannot be distinguished using qualitative criteria alone (models 2, 4, 5 and 6), are potentially distinguishable by their quantitative responses to travel time. However, some models are likely to more easily distinguished than others: the quantitative predictions of the time-limiting and reserves-limiting models are likely to differ markedly because the cost of oviposition relative to travelling is much greater when measured in eggs than in time (Ch.2); whereas the predictions of models 5 and 6 (eggs & time independently limiting and eggs & hosts independently limiting) will be very similar because oviposition time is likely to be short relative to travel time. Quantitative support for a model therefore does not necessarily exclude alternative explanations.

Interpreting Fixed and Flexible Responses.

There are at least two difficulties in interpreting the behaviour of animals from optimality models. The first is that factors assumed to be fixed constraints may in fact covary with other parameters in the model. When these factors are also traits of the study animal, this means that a behaviour that is assumed to be fixed may actually be flexible. For example, most functional models of oviposition behaviour assume that oviposition time (the interval between the laying of one egg of a clutch and the next) is independent of other model variables, such as travel time (though oviposition time is allowed to change with clutch size in some models; e.g. Parker & Courtney 1984). Rather than being a fixed constraint, oviposition time may be flexible and respond to environmental conditions. There are good intuitive reasons for predicting that this will be the case: at long travel times, fewer clutches are laid and hence it is of greater importance to females that they make correct decisions. Natural selection will therefore favour longer oviposition times _if_ these improve accuracy of the decisions.

The second difficulty in interpreting the behaviour of animals from optimality models is that the predicted responses may not occur because they are determined by the animal's genotype, rather than by prevailing conditions. Rigorous testing of specific functional models is made impossible in this situation. It should be noted that this does not imply that the responses are necessarily 'sub-optimal'; they may be optimal given the constraint that they cannot respond to the current environment.

Flexibility in response is predicted where the value of the factor: (a) varies considerably _between_ generations and (b) can be accurately estimated from experience _within_ generations. Travel time and egg-load are two factors for which a flexible response in clutch size is predicted; both may vary greatly between generations and both are potentially easy to estimate from experience. The response to future multiple oviposition will be flexible only if potential cues to its level, such as the number of females encountered during an oviposition bout, are reliable.

The assumption that responses to egg-load and seed weight are flexible is examined in detail in Chapter 4.

Aims.

The present chapter has three aims:

(1) to calculate the larval competition curve for *C. maculatus*;

(2) to determine how fitness is related to clutch size under different currency assumptions;

(3) to test quantitative predictions for the functional models developed in Chapter 2.

MATERIALS & METHODS.

Predicting Optimal Clutch Sizes.

Optimal clutch sizes for all of the models described in Chapter 2 can be determined once the relationship between total offspring fitness and either the number of eggs per seed or the amount of time spent ovipositing per seed has been determined. If the rate of egg-laying is known, then only one of the relationships need be measured.

Total offspring fitness is approximated by the number of female offspring multiplied by their potential fecundity. The present study does not include any factors relating to the fitness of male offspring in its definition of TOF and does not consider the influence of offspring phenotype on the quality of eggs they produce.

Ideally, the relationship between TOF and number of eggs per seed (the larval competition curve) should be measured directly, but this was impractical. Instead, it was measured in two parts, and these parts combined to produce the overall relationship. Experiment 3.1 measured the number and weight of females emerging as a function of the number of larvae per seed, and Experiment 3.2 measured female fecundity as a function of emergence weight.

Three more pieces of information are required before predictions can be made for all of the models: the cost of reproduction (Expt. 3.3); mean oviposition time; and the effect of travel time and clutch size on this mean (both Expt. 3.4).

Experiment 3.1. Effects of larval competition.

In order to measure the effects of larval competition on survival, sex ratio and emergence weight, a range of egg-loads were generated by placing varying numbers of recently emerged adults (about 16, 80, 130 and 200) into 400ml plastic containers with approximately 100 cowpeas. The adults were removed after one day and all the seeds kept for a week before being categorised according to the number of hatched eggs on their surface. Each seed was kept separately at 30°C and 35% rh until all adults had emerged. The date that each beetle emerged from the seed, its sex and emergence weight were noted. Egg-loads up to twenty were produced in this experiment. It should be noted that although egg loads were naturally generated, manipulated egg-loads produce similar trends (Expt. 4.8).

Experiment 3.2. Effect of female emergence weight on realised fecundity.

Fourteen females were weighed at emergence and retained in a 50ml container with a male until the female died. The pair was provided with 4 fresh seeds daily and the number of eggs laid during the previous day counted, so that the lifetime egg-production of each female could be determined. (These fourteen females constituted group 1 of Experiment 3.3).

Experiment 3.3. Cost of reproduction.

The cost of reproduction was determined by experimentally manipulating the number of eggs laid by females. This was achieved by varying the availability of mates and oviposition sites to them.

Four experimental treatments were set up: group 1 females (see Expt. 3.2) were given a single mate for the duration of the experiment and four pristine seeds each day; group 2 females were given a mate but no seeds; group 3 females were allowed to mate once, then the male removed and the female given 4 new seeds each day; and group 4 females were mated once and given no seeds on which to oviposit. The experiment was conducted at 30°C and 35% rh. Each female was checked twice daily (at 0830 and 2030h) so that longevity was determined to the nearest half day, and the number of eggs laid by each female, on both seeds and the container, was determined.

Experiment 3.4. Effect of travel time and clutch size on oviposition time.

Eighteen-hour-old virgin females were mated and two hours later given a seed on which to lay. When the female left the seed and walked at least 15mm away from it, the clutch was deemed complete and the cowpea was removed and its egg-load. Subsequent seeds were presented to females at intervals of 1, 10, 240 or 1440 miniutes, starting after the female had left the seed and ending when she mounted another. To was estimated as the total amount of time spent on a seed divided by the number of eggs laid, for the first five clutches laid.

Measuring Observed Clutch Sizes.

The clutch size models presented in Chapter 2 predict quantitatively different responses to several factors. The response of ovipositing females to the following factors was examined: host encounter rate (Expt. 3.5), number of conspecific females encountered during an oviposition bout (Expt. 3.6), current host's egg-load (Expt. 3.7), lifespan (Expt. 3.8) and residual lifespan and residual egg-complement (Expt. 3.9).

Experiment 3.5. Effect of travel time on clutch size.

Data were collected during Experiment 3.4. The experimental protocol was therefore the same as that descibed above. Clutch size was determined for the first five clutches laid by females experiencing travel times of 1 minute, 10 minutes, 2 hours or 1 day.

Experiment 3.6. Effect of conspecific females on clutch size.

The influence of conspecific females on observed clutch sizes was examined by allowing females to lay clutches in the presence of one or more other females.

Virgin females that had been isolated from conspecifics for the duration of the 15 h since their emergence were placed in 35 ml containers with single males. Two hours later (during which time most pairs had mated) test females were marked on the elytra with a small spot of acrylic paint (Rowney PVA Colour) and placed for 2 h in 35 ml holding containers with 0, 1 or 4 mated females of similar age. Test females were then moved to 35 ml test containers and presented with pristine seeds on which to oviposit. When the clutches were complete, females were returned to their holding containers, the seeds removed and their egg-loads determined. Subsequently, test females were presented with new seeds in the test container at half hourly intervals and returned to the holding containers between clutches. The sizes of each of the first five clutches were determined.

Females were presented with seeds in a test container (rather than the holding container) so that the non-test females could not oviposit on the same seeds as the test female. Non-test females were mated because females may be able to detect the reproductive state of conspecifics, and adjust their clutch size on the basis of this information.

Experiment 3.7. Effect of current egg-load on clutch size.

Seeds with egg-loads of 1, 3, and 5 were obtained by allowing newly-emerged adults to mate and oviposit in 400ml dishes containing 100 cowpeas for 24 h prior to the start of the experiment. In order to reduce the variance in clutch size attributable to differences in seed weight, the lightest and heaviest 20% of seeds were excluded from the experiment.

Young, recently mated females were presented with seeds of two different egg-loads in an alternating sequence at 30 minute intervals. The two egg-loads used in each test were 1 and 3 eggs/seed, 1 and 5 eggs/seed or 3 and 5 eggs/seed. At the end of the experiment, the final egg-load of each seed was determined and females were killed in alcohol, their elytra lengths measured and residual egg-complements determined.

Experiment 3.8. Effect of emergence weight on lifespan of virgin females.

Thirty-five newly-emerged virgin females were retained separately without mates or seeds in 50ml containers and checked twice daily to determine the time of death to within half a day. Elytra lengths were measured after death.

Experiment 3.9. Effect of female age and previous oviposition experience on clutch size.

The relative importance of residual lifespan (**T**(**t**)) and egg-complement (**E**(**t**)) on clutch size was determined by varying the length of time that individual females were retained without oviposition sites and then presenting all females with seeds at a similar rate.

Mated females were retained singly for 1-8 days before being presented with a single seed on which to oviposit. When the clutch was complete, the seed was removed, its egg-load determined and the female again retained in isolation. Subsequent seeds were generally given to females at daily intervals, but females given their first seed when they were 5, 6, or 7 days old were given a second seed immediately after their first and then dissected to determine their residual egg-complements.

Comparing Observed and Predicted Clutch Sizes.

Curve fitting.

The goodness of fit between observed clutch sizes and those predicted by the different models was initially assessed by eye. The quantitative fit of the best of the models was then determined using the NLIN non-linear least squares regression procedure of the SAS statistical package (SAS Institute Inc. 1985). This procedure was also used to fit parameter values to the different offspring *per capita* fitness functions.

RESULTS

Experiment 3.1. Effects of Larval Competition.

Number of survivors.

The number of adults emerging from a seed was a non-linear function of the number of larvae present (see Fig. 3.1(a)). Models based on linear and exponential *per capita* fitness functions (Ch.2) explained similar amounts of variance in the number of survivors (about 90%, see models II and III, Table 3.1(a)), and both of these models explained significantly more of the variance than the density-independent model (model I: $S(N) = dN$; $P < 0.001$). The non-linear convex-upwards model (model IV) explained less of the variance in the number of survivors than the density-independent model, because the *per capita* fitness function is convex-upwards, whereas the survivorship function is convex-downwards (Fig. 3.1(b)).

The two survival models based on linear and exponential *per capita* fitness functions both peaked at around 16 eggs/seed (Table 3.1(a)). The lack of data at egg-loads greater than twenty makes it impossible to distinguish between these two functions.

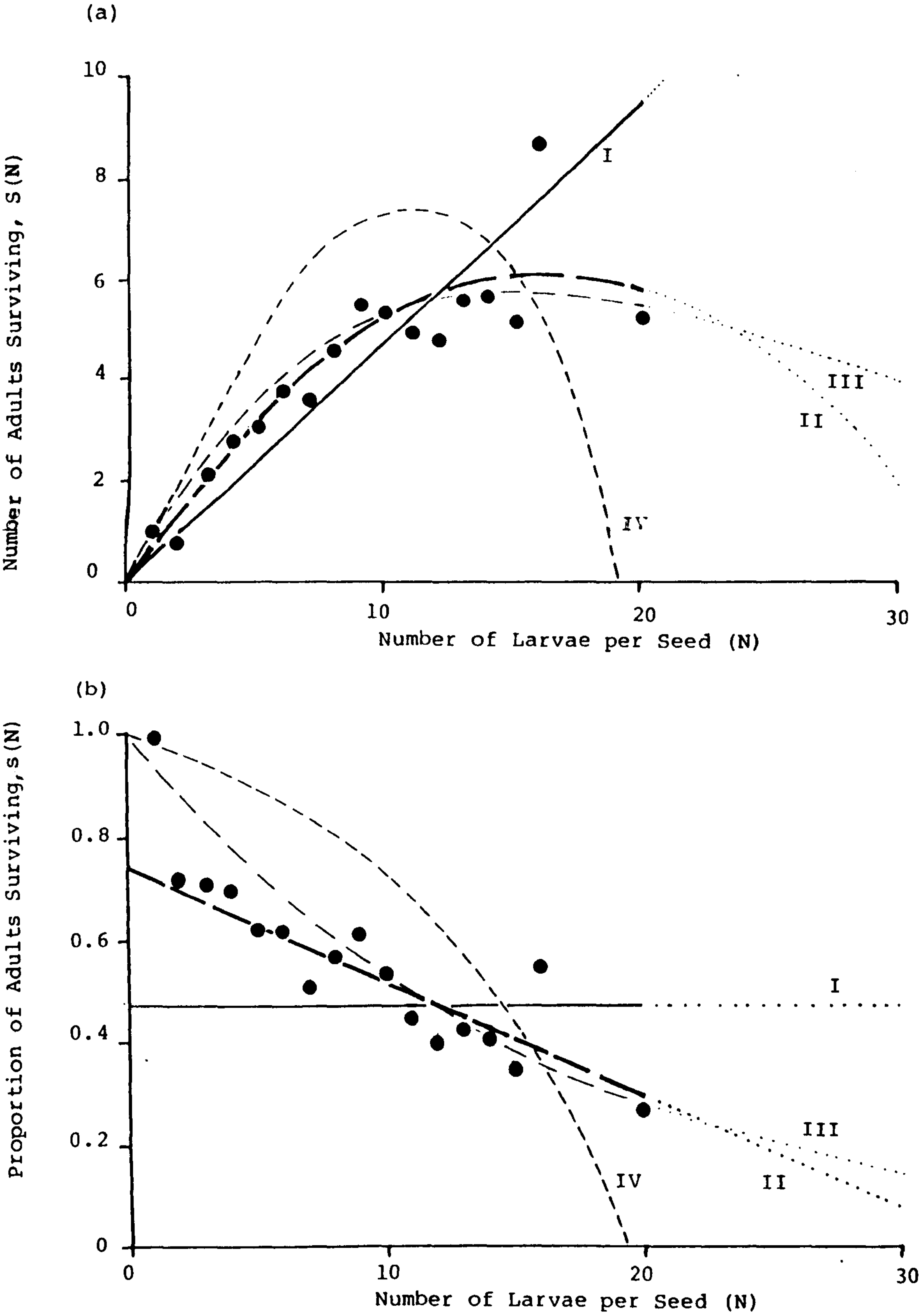


Figure 3.1 Relationship Between Number and Proportion of Adults Surviving and Number of Larvae per Seed.

(a) Number of adults emerging; (b) proportion of adults emerging. Roman numerals refer to models I-IV in Table 3.1. Note that for clarity standard error bars have been left off the figure. Sample sizes for each point are given from left to right: 15, 25, 23, 25, 24, 24, 9, 9, 8, 12, 9, 9, 11, 7, 5, 5, 3.

MODEL	PARAMETER VALUES (± approx SE(1)) 1st	2nd	r^2(x100)	RSS	RMS	N*
a. No. survivors =						
Ia. dN	0.4763 ± 0.0125		86.7	500.9	2.246	-
IIa. N(a-bN)	0.7361 ± 0.0318	0.0220 ± 0.0025	90.0	373.6	1.683	16.7
IIIa. $N(e^{-cN})$	0.0651 ± 0.0022		89.4	398.1	1.789	15.4
IVa. $N(1-cN^2)$	0.0026 ± 0.0001		72.9	1015.4	4.553	11.3
b. Total offspring Fitness(2) =						
Ib. dN	11.7618 ± 0.3427		84.1	374760	1680	-
IIb. N(a-bN) (3)	20.3104 ± 0.8001	0.7254 ± 0.0638	89.9	236890	1067	14.0
IIIb. $aN(e^{-cN})$ (3)	23.3878 ± 1.2883	0.0613 ± 0.0053	90.1	233716	1053	16.3
IVb. $N(a-cN^2)$ (3)	16.2638 ± 0.5175	0.0286 ± 0.0028	89.3	252419	1137	13.8

Table 3.1 Estimated Parameter Values for Several Functions Describing the Relationship Between the Number of Survivors or Total Offspring Fitness and Clutch Size.

All parameter values calculated using non-linear least-squares regression procedures (SAS Inc. 1985). RSS = residual sum of squares; RMS = residual mean square; (1) see text for explanation; (2) number of female survivors multiplied by their potential fecundities (see text); (3) parameter a is included as a scaling constant.

Adult sex ratio.

The sex ratio of emergent adults was independent of egg-load ($F_{1,221} = 0.01$, $P > 0.9$); 53.6% of emerging adults were female.

Emergence weight.

Adult emergence weight declined linearly with the number of larvae per seed (Fig. 3.2). The rate of reduction in emergence weight was greater for females than males, but not significantly so (comparison of slopes: $t = 1.08$, df = 222, $P > 0.2$). The relationship between the number of larvae per seed (**N**) and female emergence weight (**W**) was described by the function:

$$\mathbf{W}(\mathrm{N}) = 4.86 - 0.04\ \mathbf{N} \qquad [3.1]$$

Experiment 3.2. Effect of Female Emergence Weight on Lifetime Egg Production.

Lifetime egg production (**E**) of mated females given 4 seeds per day increased linearly with female emergence weight. The relationship was described by the function:

$$\mathbf{E}(\mathbf{W}) = -31.18 + 17.49\ \mathbf{W} \qquad [3.2]$$

In other words, lifetime egg production increased by approximately 17 eggs for every increase of 1 mg in emergence weight (Fig. 3.3). The effect of larval

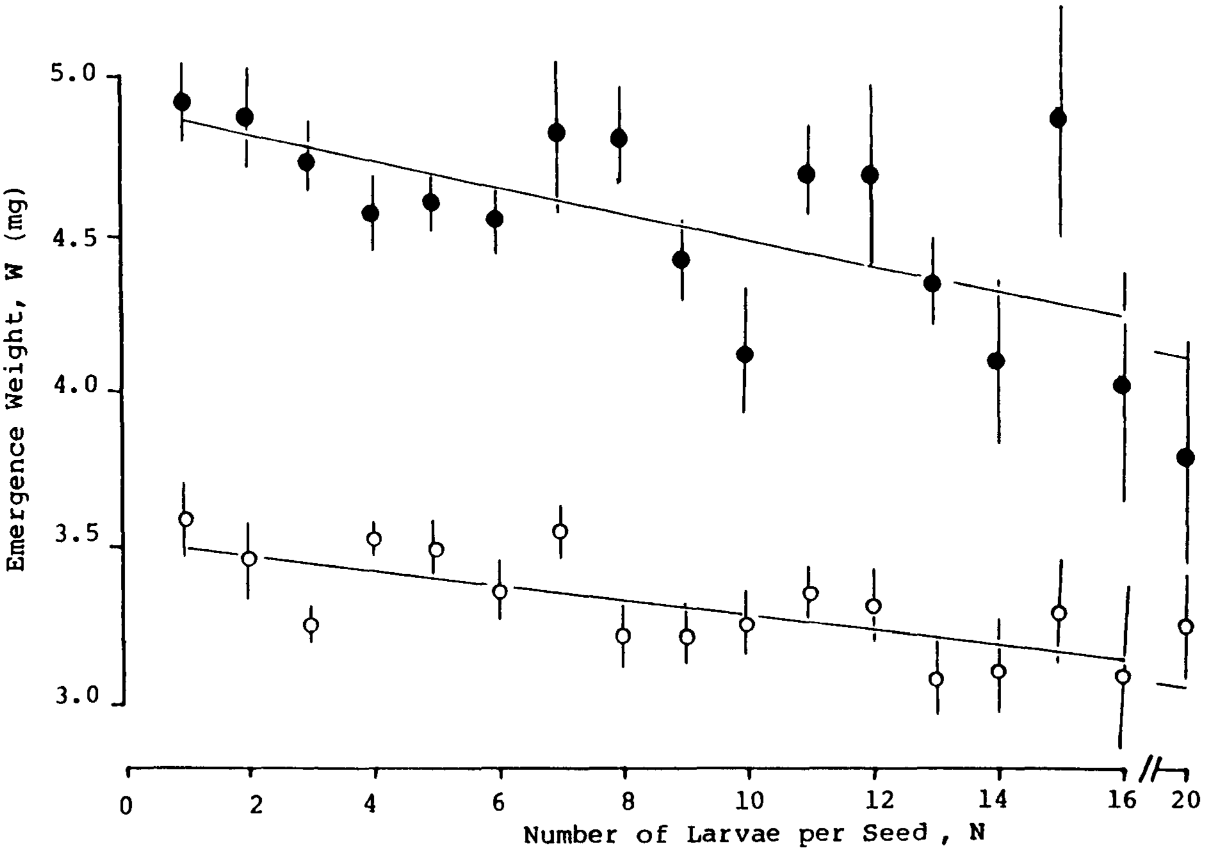


Figure 3.2 Effect of Larval Crowding on Emergence Weights of Male and Female *C. maculatus*.

Regression equations:
Females (●): $W = 4.86 - 0.04\ N$; $F = 12.04$, df = 1,188, $P < 0.001$
Males (○): $W = 3.53 - 0.02\ N$; $F = 13.21$, df = 1,176, $P < 0.001$

Vertical bars are standard errors. For sample sizes see Fig. 3.1.

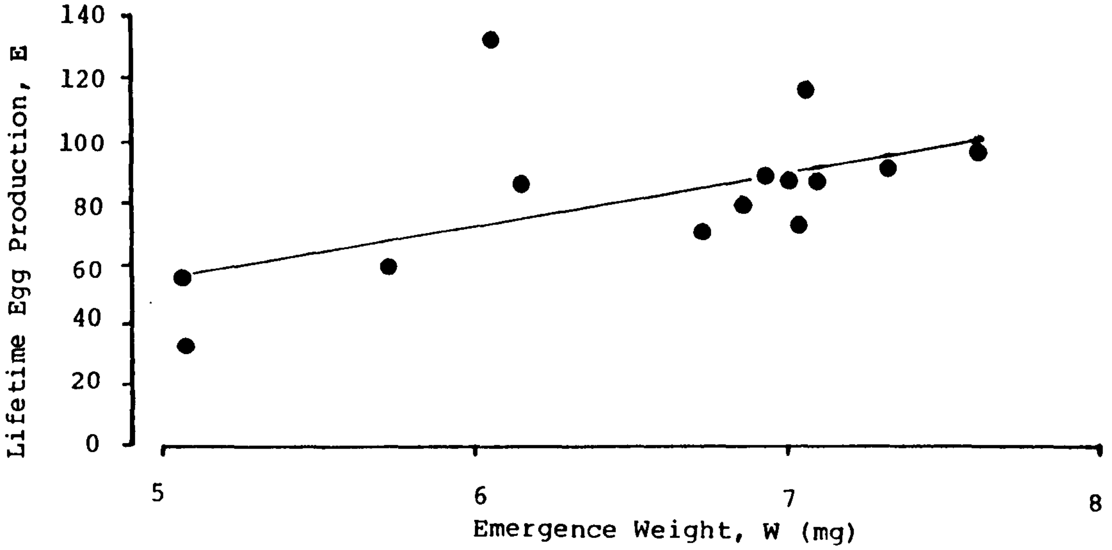


Figure 3.3 Relationship Between Lifetime Egg Production and Female Emergence Weight.

Regression equation: $E = -31.18 + 17.49\ W$; $F = 5.04$, $df = 1,13$, $P < 0.05$.

competition on <u>per capita</u> fecundity was estimated by substituting Equation 3.1 into Equation 3.2. Fecundity as a function of the number of larvae per seed is described by the function:

$$E(N) = 53.82 - 0.70 N \qquad [3.3]$$

Constructing the Larval Competition Curve.

Total offspring fitness of larvae from single seeds was estimated by multiplying the observed number of adult females emerging by their predicted fecundities (using Eqn. 3.3). The three density-dependence functions (and the density-independence function) were then fitted to this manufactured data. The resultant LCC and offspring <u>per capita</u> fitness curve are shown in Figure 3.4.

The shape of the LCC was not very different from the curve describing the number of survivors at different larval densities. All 3 density-dependence functions explained similar amounts of variance in TOF and peaked at clutch sizes between 14 and 16 (Table 3.1(b)). As stated earlier, the lack of data for egg-loads greater than 20 eggs/seed means that it is not possible to distinguish between the different fitness functions.

Experiment 3.3. Cost of Reproduction.

Figure 3.5 illustrates the cost of reproduction. The

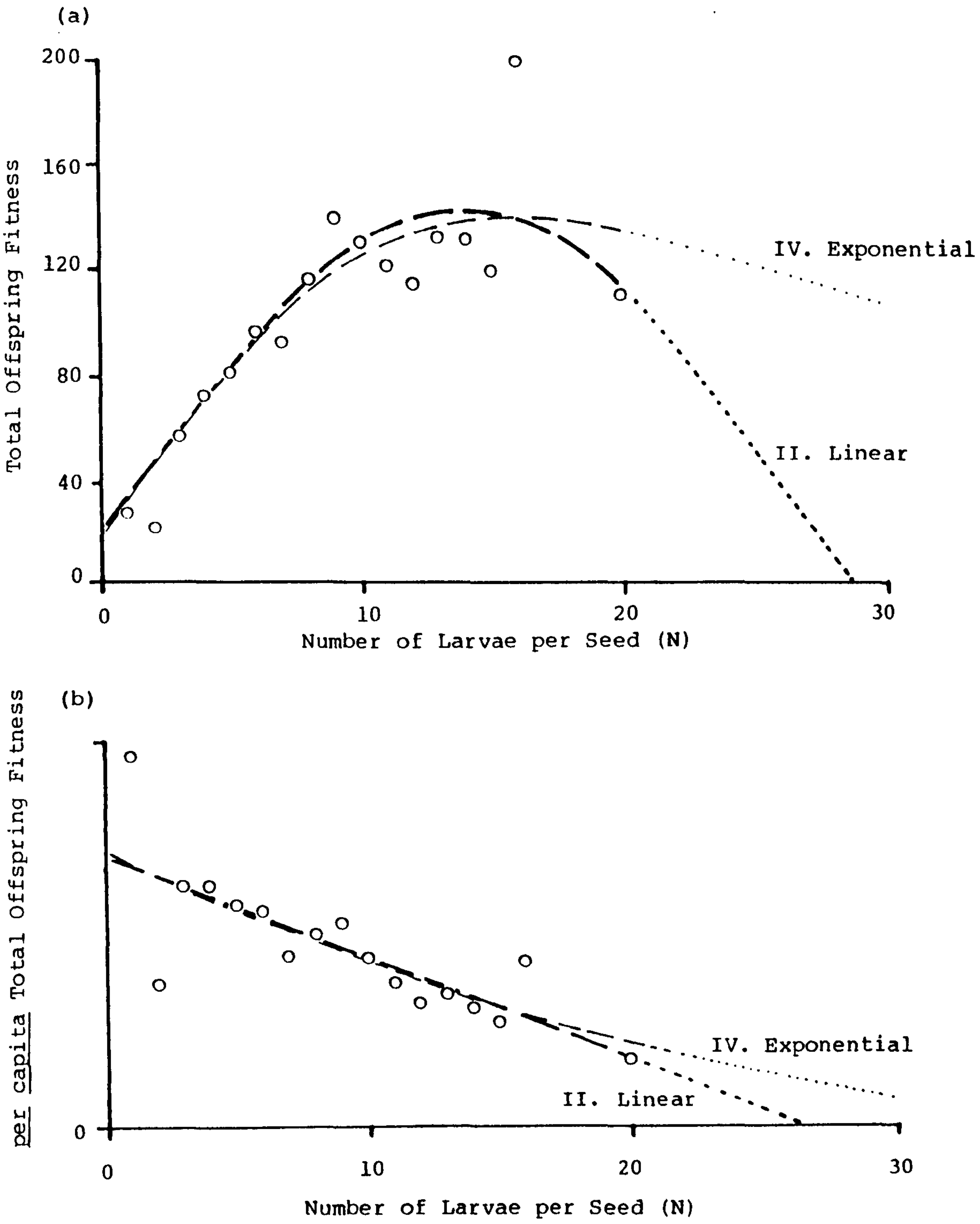


Figure 3.4 Relationships Between Total Offspring Fitness and *per capita* Total Offspring Fitness, and Number of Larvae per Seed.

(a) Total offspring fitness (number of females emerging x their potential fecundity (eqn. 3.3, text)); (b) *per capita* TOF. See legend to Fig. 3.1 for details of sample sizes. Roman numerals refer to models in Table 3.1(b). Note that figure (a) is also referred to as the Larval Competition Curve or Number-Fitness Relationship.

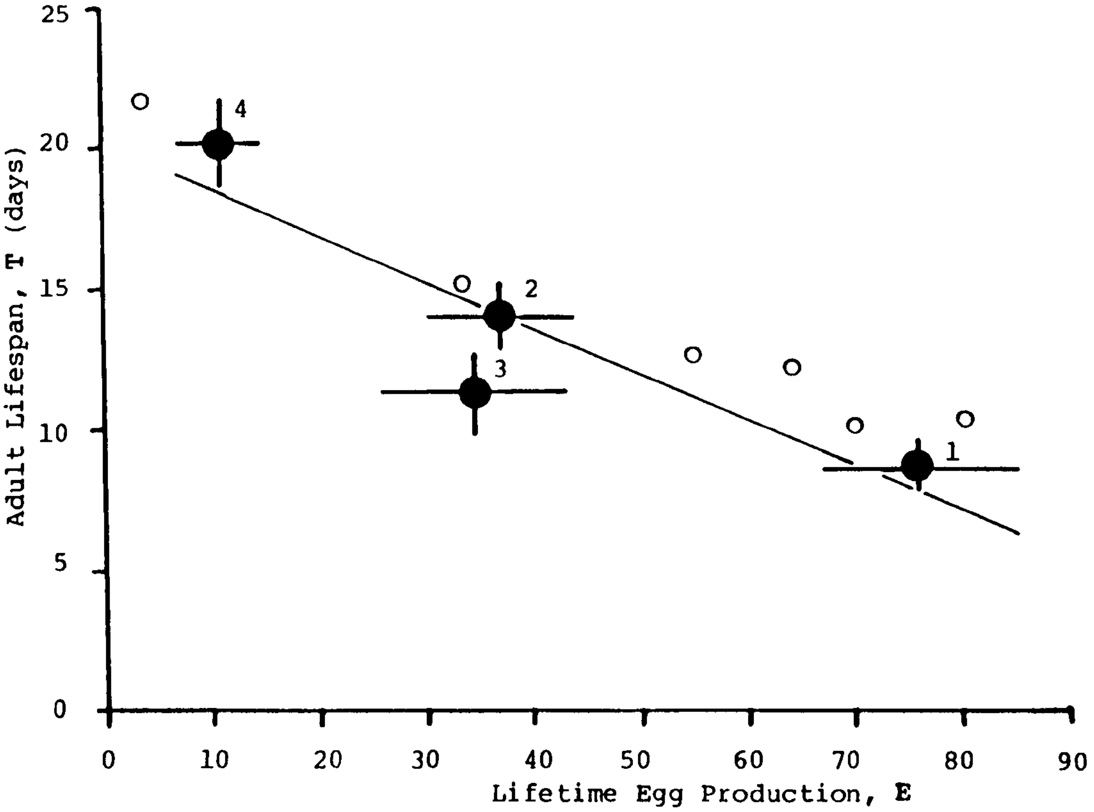


Figure 3.5 The Cost of Reproduction in *C. maculatus*: Relationship Between Adult Lifespan and Lifetime Egg Production.

Solid circles are means (± standard errors) obtained during Expt. 3.3. Regression on means: $T = 20.18 - 0.16\ E$. Figures refer to group numbers. Group 1: male retained after day 1, seeds given each day; Group 2: male retained, no seeds given; Group 3: seeds given each day, male removed after day 1; Group 4: male removed after day 1, no seeds given. Sample sizes: 14, 15, 14 and 15, respectively. Open circles are from El-Sawaf (1956), see text.

regression coefficients for between- and within-groups were calculated using the methods described by Snedecor & Cochran (1978, pp. 436-438). The between-groups regression coefficient was negative and equal to -0.1643, whilst that for within-groups was positive and equal to 0.0676. In other words, within each group, some females lived longer and laid more eggs, but each egg that was laid cost, on average, 0.16 days of life (*c.* 3.8 hours). A linear regression on the means for the four groups gave a similar regression coefficient (-0.1629 ± 0.0247, SE), and this was significantly different from zero ($t = 6.595$, $df = 3$, $P < 0.01$). Note that the opposite regression, with fecundity as the dependent variable and lifespan as the independent variable, yielded a slightly different conversion rate (1 egg cost 0.1917 days of life).

In his thorough study of the factors influencing longevity, oviposition and development in *C. maculatus*, El-Sawaf (1956) manipulated the number and size of cowpeas he gave to females and measured their subsequent lifespan and fecundity (see pp 71-79). Re-analysis of this data suggests that a similar cost of reproduction was exhibited by El-Sawaf's animals. The regression coefficient (for the regression of mean lifespans on mean fecundities) was -0.1492 ± 0.0073 SE, suggesting that each egg 'cost' 3.4 hours of life. This regression coefficient was not significantly different from that calculated in the present study ($t = 0.291$, $df = 8$, NS; Fig. 3.5).

Experiment 3.4. Effect of Travel Time on Oviposition Time.

Oviposition time was calculated by dividing the amount of time a female spent on each seed by the size of the clutch she laid. Median oviposition times increased as the time between successive seed encounters, **Tt**, increased (Fig. 3.6). Within each treatment group, **To** was relatively constant over successive clutches, but there was a tendency for females given seeds at daily intervals to take longer over later ovipositions ($r_s = 0.343$, $n = 25$, $0.05 < P < 0.1$).

Oviposition time was independent of the number of eggs laid in a clutch. This was true for all females laying their first clutch ($r_s = -0.189$, $n = 50$, NS) and for females that were laying their second clutch up to 10 minutes after their first ($r_s = 0.048$, $n = 31$, NS). Total oviposition time divided by the number of eggs laid therefore appears to be an accurate approximation of **To**.

The importance, in evolutionary terms, of the positive correlation between **To** and **Tt** is not immediately obvious: it may reflect an adaptive response by the female to differences in the perceived value of seeds encountered at different rates, or it may be the result of constraints associated with ageing. Whatever the cause, the variation in oviposition time associated with **Tt** must be included in the tests of the functional models that follow.

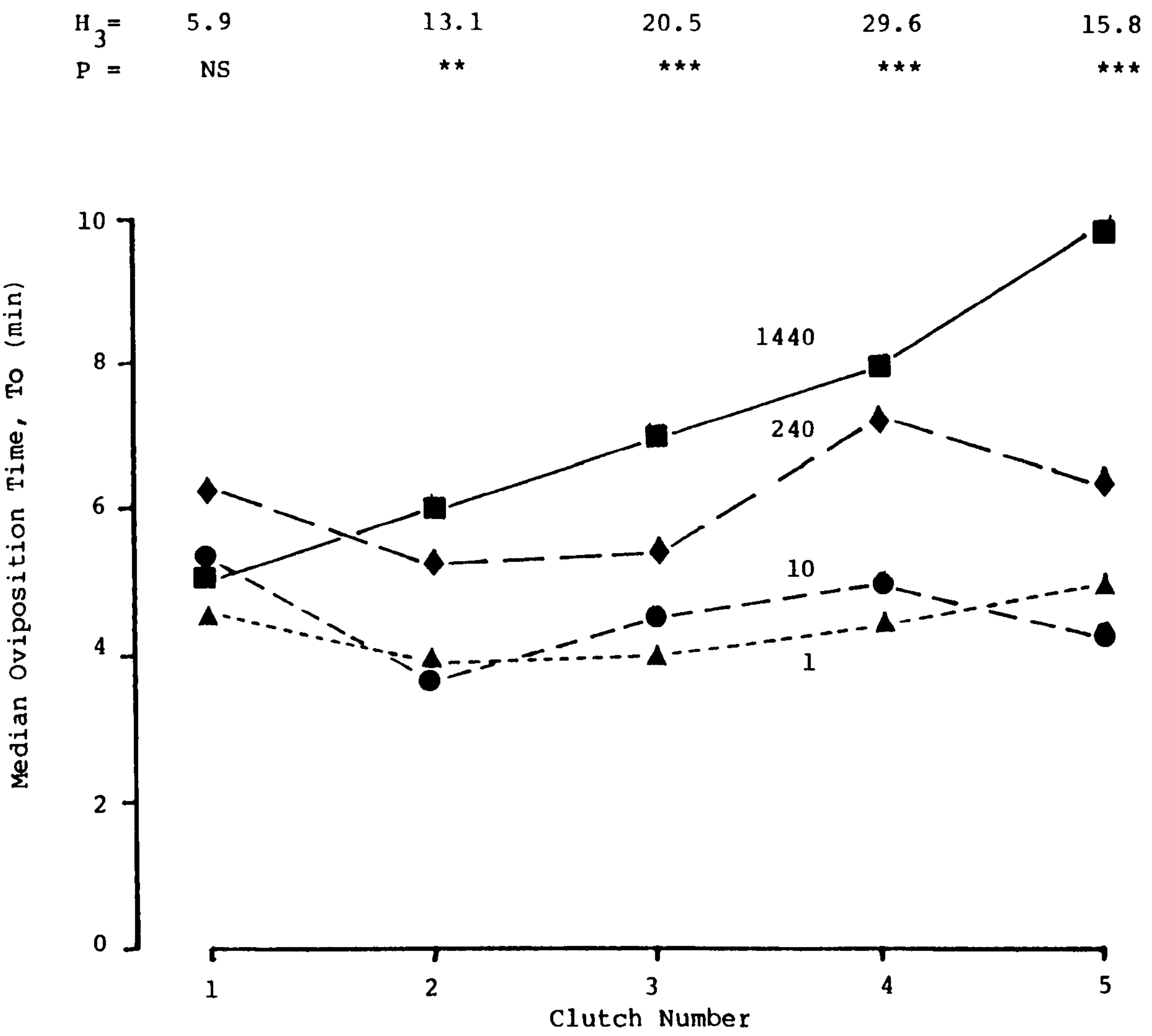


Figure 3.6 Relationship Between Median Oviposition Time and Clutch Number at Various Travel Times.

Travel times: 1440 min (■), n=5 females; 240 min (♦), n=14; 10 min (●), =15; 1 min (▲), n=16. Statistics above figure are Kruskal-Wallis H statistics for differences between treatments and its associated probability value. NS not significant, ** P<0.01, *** P<0.001. Oviposition time differs between treatments except on clutch 1.

Predicting Optimal Clutch Sizes.

Model parameters.

The parameter values used in the clutch size models are listed in Table 3.2, and are derived from the experiments described above. Where parameters were observed to exhibit considerable variation, the extreme values of their range were entered into each model and the associated predictions compared. For example, median oviposition time varied between 3.6 min and 10.0 min over the first five clutches, depending on the value of **Tt** (Fig. 3.6). Therefore, these two values of **To** were entered into the models and the effect on $\mathbf{N}^{\wedge}$ observed.

The range of values for **T** (female lifespan), was determined from the mean values for females given a mate and oviposition sites and those denied access to both of these (see Fig. 3.6). The range of values for **E** (lifetime egg production) was determined from the mean number of eggs laid by females given continuous access to mates and seeds and that by mated females denied access to seeds. (This will tend to underestimate the smallest value of **E**.) The range of values for **T/E** (the amount of time available to lay each egg) was determined by the ranges of **E** and **T**. The predicted clutch size when eggs and hosts are limiting is dependent on the ratio E/H. In the present study, the number of seeds

PARAMETER	RANGE USED IN MODELS
To (time taken to lay each egg)	3.6 - 10.0 min
T (total time available for laying)	8.5 - 20.0 days (12240 - 28800 min)
E (total number of eggs available)	40 - 80 eggs
T/E (time available for laying each egg)	153 - 720 min/egg
N* (the most productive clutch size)	15 eggs/seed
s(N) (survival or fitness function)	a-bN and e^{-cN}
CR (conversion rate)	1 min = 0.0026 - 0.0122 eggs

Table 3.2 Parameter Values Used in Clutch Size Models.

See text for method of derivation and Table 2.1 for key to symbols used.

available to the female was controlled by adjusting the travel time, **Tt**. If time is finite and equal to **T**, then **H** can be approximated by **T/Tt** and **E/H** by **E.Tt/T.** The values of **a**, **b**, and **c** used to describe the per capita fitness functions were such that **a/2b** and **1/c** were equal to 15. The range used for the conversion rate between eggs and time was the 95% confidence limits for the regression coefficient for the regression of longevity on fecundity.

Predictions

The values of **Tt** (travel time) included in the model were 1, 10, 240 and 1440 minutes and the values of **i** (number of ovipositing females) were 1, 2, 5, and 10. Current egg-load, **x**, was set to zero for all models. The predictions of the single oviposition models are presented in Table 3.3, and those for the multiple oviposition in Table 3.4.

Of the single oviposition models that predict an increase in clutch size with travel time, only the time limiting model predicts clutch sizes of greater than 1 egg/seed at travel times of less than 10 minutes. This model predicts that at travel times of 1 day clutch sizes will approximate the most productive clutch size (15 eggs/seed). The reserves limiting model predicts a gradual increase in clutch size with travel time, with clutch sizes estimated at 1-7 when seeds are encountered at 2 h intervals, and 4-11 when they are encountered at daily intervals. The eggs &

MODEL	PARAMETER VALUES	TRAVEL TIME, Tt (min)			
		1	10	240	1440
1. Hosts		15	15	15	15
2. Time	Linear; To = 3.6	3	7	14	15
	= 10.0	2	5	12	14
	Exponential; To = 3.6	2	5	13	14
	= 10.0	1	3	10	14
3. Eggs		1	1	1	1
4. Reserves	Linear; CR = 0.0026	1	1	1	4
	= 0.0122	1	2	7	11
	Exponential; CR = 0.0026	1	1	3	6
	= 0.0122	1	1	5	10
5. Eggs & Time	Linear; T/E = 153	1	1	1	10
	= 720	1	1	1	2
	Exponential; T/E = 153	1	1	2	10
	= 720	1	1	1	2
6. Eggs & Hosts	Linear; T/E = 153	1	1	1	9
	= 720	1	1	1	2
	Exponential; T/E = 153	1	1	2	9
	= 720	1	1	1	2
Observed Clutch Sizes		2.3	3.0	4.0	5.5

Table 3.3 Quantitative Predictions of Clutch Size Models: Single Oviposition.

Predicted values are given as the integer with the highest associated fitness return. Parameter values not included in column 2 do not substantially alter the predictions of the model. See Table 3.2 for list of parameters included in the models.

MODEL		NUMBER OF OVIPOSITING FEMALES (i)											
		i=2				i=5				i=10			
		TRAVEL TIME (Tt, min)											
		1	10	240	1440	1	10	240	1440	1	10	240	1440
1.Hosts	L	8				3				2			
	E	8				3				2			
2.Time	L	1-2	3-4	7	7	1	2	3	3	1			
	E	1	2-3	6-7	7	1	1-2	3	3	1			
3.Eggs	L	1				1				1			
	E	1				1				1			
4.Reserves	L	1	1	2	5	1	1	1	2	1			
	E	1	1	2-3	4-6	1	1	1-2	2-3	1			
5.Eggs & Time	L	1	1	1-2	5-7	1	1	1	2-3	1			
	E	1	1	1-2	2-7	1	1	1	2-3	1			
6.Eggs & Hosts	L	1	1	1-2	5-8	1	1	1	2-3	1	1	1	2
	E	1	1	1-2	2-8	1	1	1-2	2-4	1	1	1-2	2

Table 3.4 Quantitative Predictions of Clutch Size Models: Multiple Oviposition.

See notes for Table 3.3. (a) L = linear fitness function used; E = exponential fitness function used.

time limiting and the eggs & hosts limiting models make virtually the same predictions: optimal clutch sizes are 1 or 2 eggs/seed until travel times approach 1 day, when they increase to between 2 and 10 eggs/seed. Increasing the number of ovipositing females decreases the predicted clutch sizes of all the models. When the number of ovipositing females is equal to 10, all of the models predict clutch sizes of 1 or 2.

Comparing Observed and Predicted Clutch Sizes.

Experiment 3.6. Effect of Travel Time on Clutch Size.

As expected, the size of the first clutch was independent of treatment group (because all females were of similar age and oviposition experience) and was equal to 3.54 ± 0.33 eggs/seed (n=50; Fig. 3.7). The size of the following 4 clutches was positively correlated with travel time (Fig. 3.8; see also legend to Fig. 3.7). However, this correlation disappeared after clutch 5, due primarily to a decline in the size of clutches laid by females given seeds at intervals of one day. A functional explanation for this decline is not immediately obvious.

There was a negative correlation between body size and the size of the first clutch (Fig. 3.9). Although this trend persisted into the second clutch, the correlation was not significant and only females given seeds at 1 minute

H_3 =	3.8	11.8	24.0	13.3	17.5	9.9	-	-
P =	NS	**	***	**	***	*	-	-

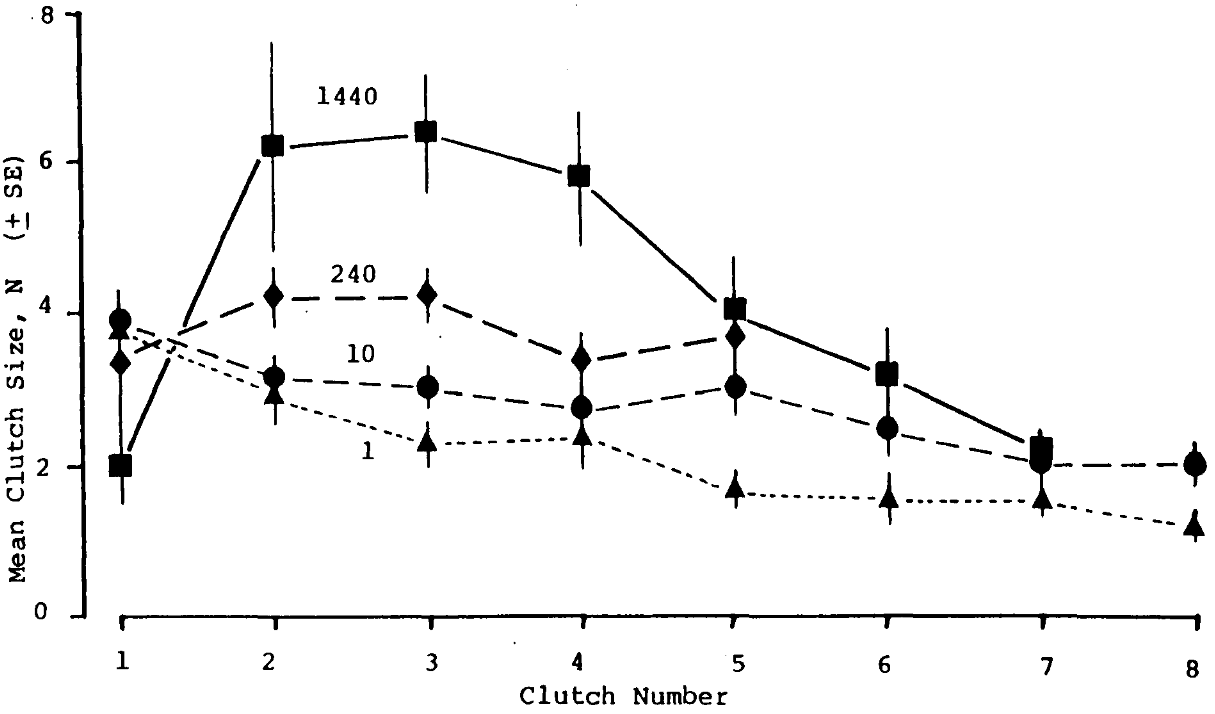


Figure 3.7 Relationship Between Mean Clutch Size and Clutch Number at Various Travel Times.

Travel times as for Fig. 3.6. Kruskal-Wallis H statistics for differences in clutch size between treatments are given above figure. NS not significant ** P<0.01, *** P<0.001. Mean clutch size differed between travel times, except on clutch 1.

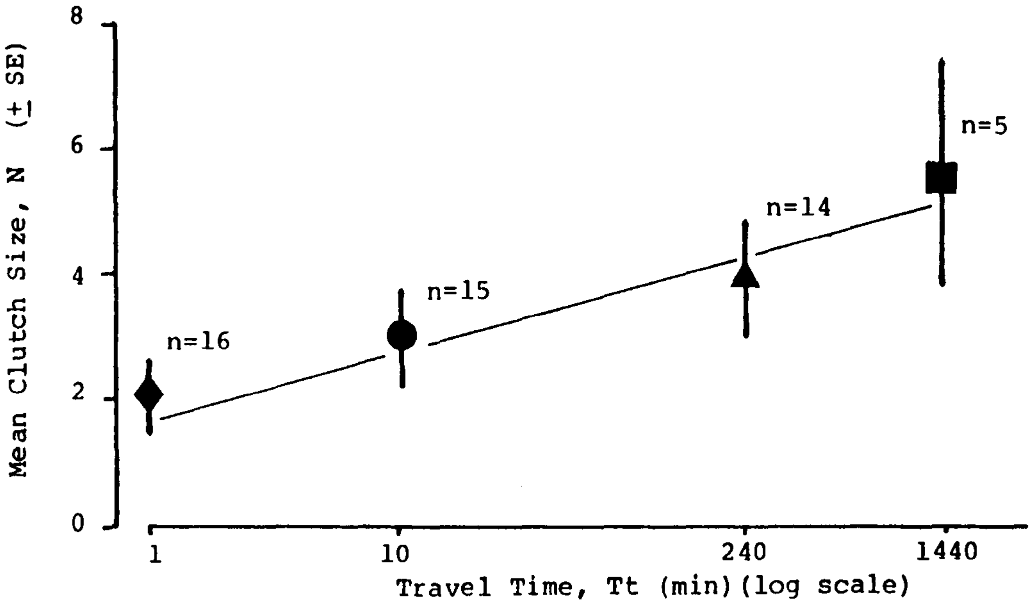


Figure 3.8 Relationship Between Mean Clutch Size and Travel Time.

Means calculated from clutches 2-5 of each female. Regression equation: **N** = 1.61 + 1.11 (log **Tt**); F = 98.2, df = 1,47, P 0.001. r^2 = 0.67.

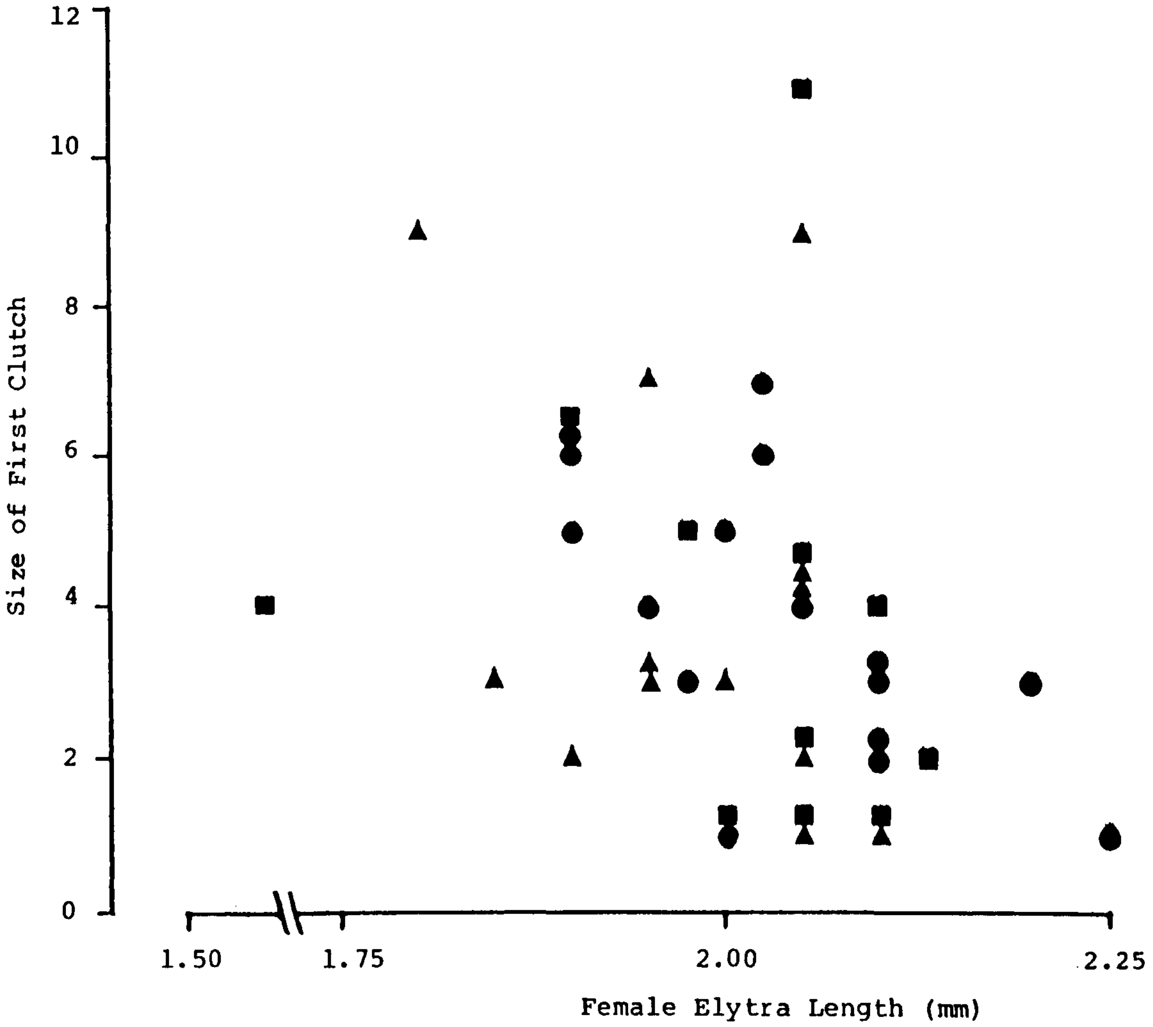


Figure 3.9 Relationship Between Size of First Clutch and Female Elytra Length

Tt = 1 min (●): $r_s = -0.605$, $n = 16$, $P < 0.02$,

Tt = 10 min (▲): $r_s = -0.171$, $n = 14$, $P > 0.2$,

Tt = 240 min (■): $r_s = -0.429$, $n = 10$, $0.1 < P < 0.2$,

All data : $r_s = -0.448$, $n = 40$, $P < 0.002$.

Note that sample sizes are slightly smaller than in Figs. 3.6 & 3.7 because the elytra lengths of 5 females were not measured.

intervals showed a significant correlation over the first five clutches (see Table 3.5).

Comparison with predictions of the single oviposition models.

The positive correlation between clutch size and Tt is in qualitative agreement with models 2, 4, 5 and 6, but not with models 1 and 3, which predict that clutch size will be independent of Tt. The hosts-limiting and eggs-limiting models can therefore be rejected and will not be discussed further. None of the remaining single oviposition models fit the data well: the reserves, eggs & time, and eggs & hosts models fit reasonably well at large values of Tt, but fail at shorter travel times, whilst the time-limiting model fails at long travel times (Table 3.3).

Comparison with predictions of the multiple oviposition models.

Inspection of Table 3.4 indicates that models 4-6 again fail to fit the data when search times are short (predicted clutch sizes never exceed 1 egg/seed at $Tt < 10$ min). However, when the number of females laying on each host (i) is equal to between 2 and 5, the time-limiting model fits the data well.

Non-linear least squares regression analysis indicates

TRAVEL TIME Tt (min)	CLUTCH NUMBER					
	1	2	3	4	mean 2-5	mean 5-8
1	-0.605 (16) [0.015]	-0.138 (16) [0.60]	-0.248 (16) [0.38]	-0.358 (16) [0.20]	-0.729 (16) [0.002]	-0.005 (15) [0.90]
10	-0.171 (15) [0.56]	-0.230 (14) [0.45]	0.034 (14) [0.90]	0.075 (14) [0.80]	-0.046 (14) [0.90]	0.225 (14) [0.45]
240	-0.429 (14) [0.13]	-0.185 (10) [0.65]	0.463 (10) [0.20]	-0.104 (10) [0.80]	0.280 (10) [0.46]	
All	-0.448 (45) [0.002]	[0.70(a)]			[0.09(a)]	

Table 3.5 Spearman's Rank Correlation Coefficients Between Clutch Size and Female Elytra Length.

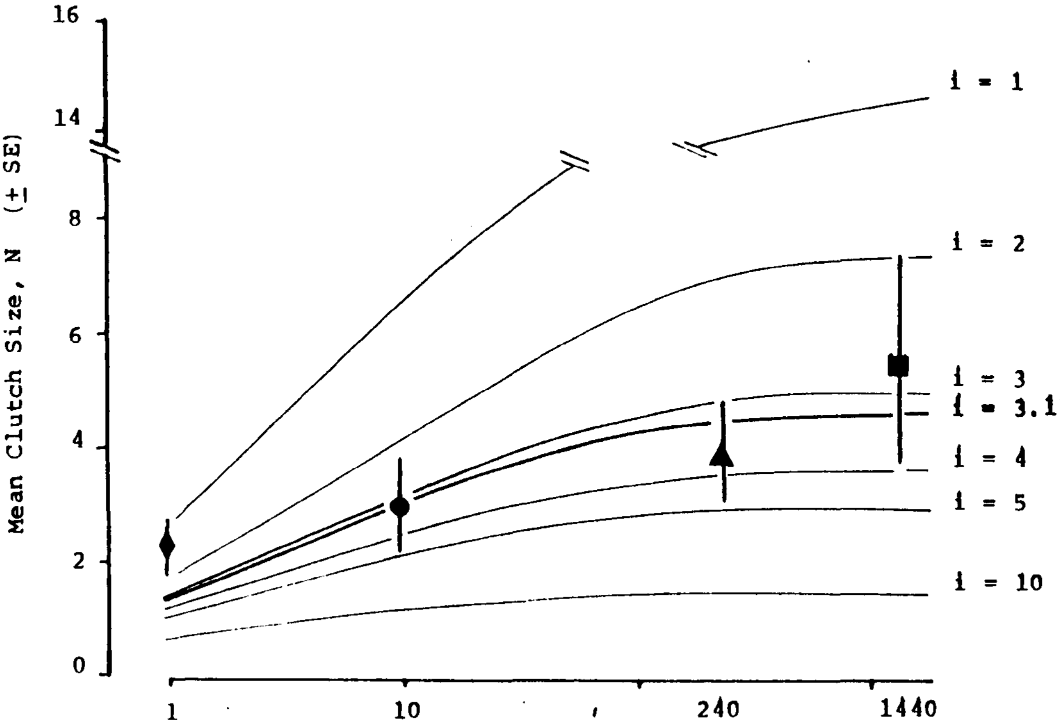
Numbers in parentheses indicate number of females used in analysis. Numbers in square brackets indicate associated probability value, calculated by interpolation of tabulated values. (a) calculated using Fisher's Combined Probability Test (Sokal & Rohlf 1981, pp 779-782).

that, regardless of whether the per capita fitness function was linear or exponential, a value of **i** equal to approximately 3.1 provided the best fit between observed clutch sizes and those predicted by the time-limiting models (Fig. 3.10; t-test on difference between values for **i** calculated using linear and exponential fitness functions: t = 0.247, df = 96, NS). The statistical model based on the linear fitness function explained slightly more of the variance in clutch size than that based on the exponential function (r^2 = 0.89 and 0.86, respectively).

Experiment 3.6. Effect of Conspecific Females on Clutch Size.

Females given seeds at half-hourly intervals showed a decline in clutch size over clutches, irrespective of whether or not other females were present (Fig. 3.11). A general linear model (GLM), which included clutch number as a covariate, indicated that the number of females present did not have a significant influence on clutch size (GLM: test of interaction effect: $F_{2,131}$ = 0.216, NS; test of treatment effect: $F_{2,133}$ = 2.03, NS; see Appendix 2 for explanation of GLM). This result is verified by a series of non-parametric tests (see legend to Fig. 3.11).

(a) Linear Fitness Function.



(b) Exponential Fitness Function.

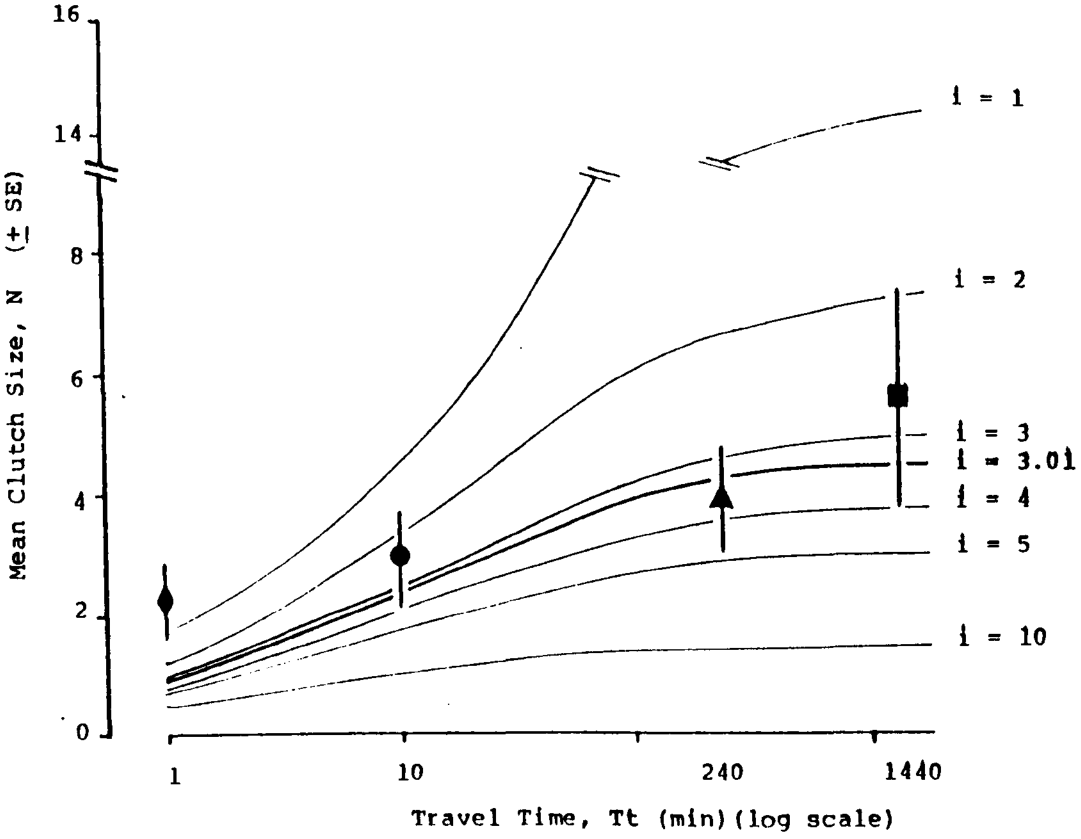


Figure 3.10 Comparison Between Observed Clutch Sizes at Various Travel Times and Those Predicted by the Time Limiting Multiple Oviposition Model with a Linear or Exponential Fitness Function.

Number of females ovipositing = i. Best fitting models, as determined by non-linear least squares regression: (a) i = 3.17 ± 0.44 (SE); F = 400.2, df = 1,49, P<0.001; RSS = 69.1. (b) i = 3.01 ± 0.46 (SE); F = 319.5, df = 1,49, P<0.001; RSS = 83.4.

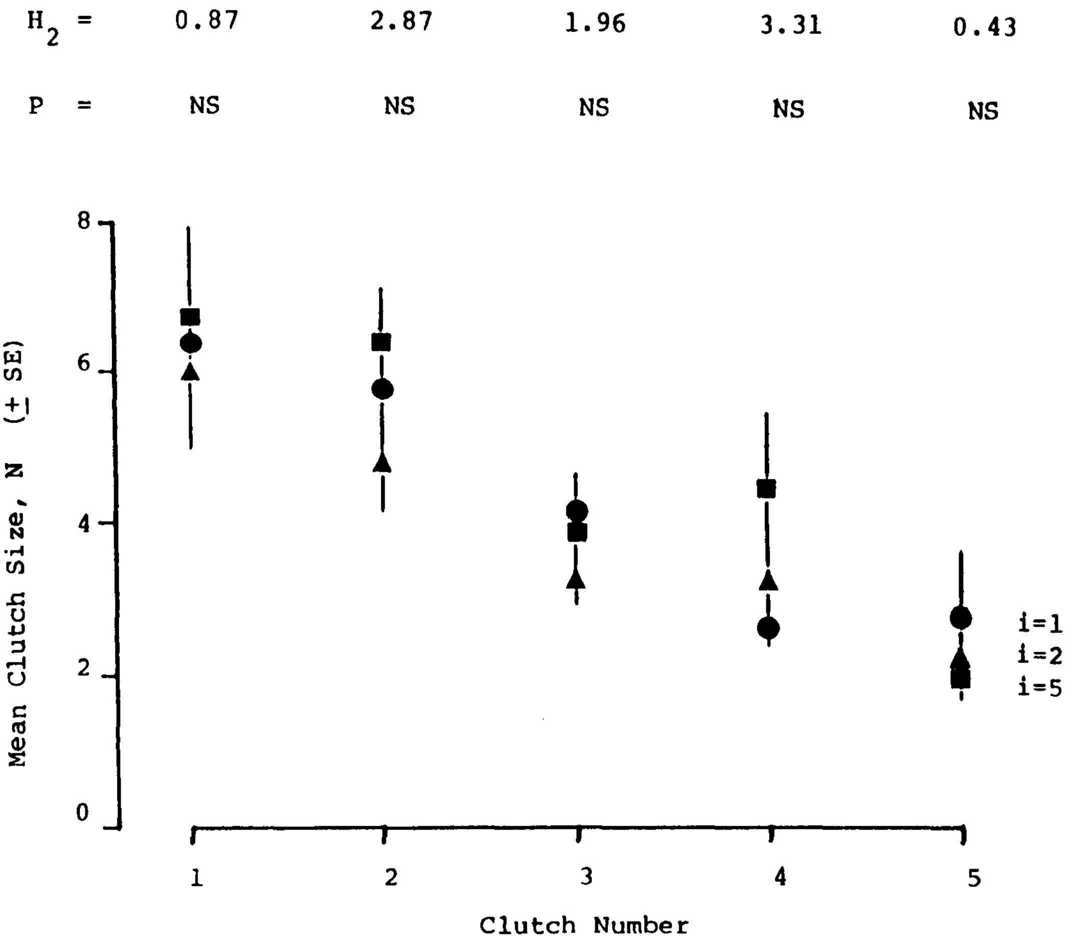


Figure 3.11 Relationship Between Clutch Size and Clutch Number when the Number of Females Encountered Prior to Oviposition Varies.

i = 1 female (●), n = 11;
i = 2 females (▲), n = 13;
i = 5 females (■), n = 9.

GLM: No. of females: F = 2.04, df = 2,131, NS; No. females x clutch number interaction: F = 0.22, df = 2,130, NS.

Kruskal-Wallis H statistics for differences between treatments at each clutch number are given above the figure. The number of females encountered prior to oviposition did not affect clutch size.

Experiment 3.7. Effect of Current Egg-Load on Clutch Size.

Clutch size declined over successive clutches, but was relatively constant for clutches 1-4. Therefore, mean clutch sizes were determined using these clutches only. As predicted by most of the functional models (when the per capita fitness function intercepts the abscissa at some finite point), clutch size was negatively correlated with the egg-load of the current seed ($r_s = -0.293$, $n = 86$, $P < 0.001$; Table 3.6).

Comparison with predictions of the time-limiting models.

Comparison between observed clutch sizes and those predicted by the time-limiting model suggests that **i** lies between 3 and 4 (Table 3.6). Whilst this value is similar to that calculated for egg-free seeds (Expt. 3.6), extreme caution should be exercised in interpreting this result because the TLMO model predicts the optimal solution given that the solution is an ESS. Although this doesn't exclude the possibility of females responding to prevailing levels of multiple oviposition (as indicated by egg-load), the same value for i on both egg-free and egg-laden seeds is not necessarily predicted.

NUMBER OF OVIPOSITING FEMALES, i	CURRENT EGG-LOAD, x		
	1	3	5
1	6.8 - 9.3	6.5 - 8.8	6.2 - 8.3
2	4.2 - 5.5	4.0 - 5.2	3.8 - 4.8
3	3.2 - 3.9	3.0 - 3.7	2.8 - 3.5
4	2.5 - 3.1	2.4 - 2.9	2.3 - 2.7
5	2.1 - 2.5	2.0 - 2.4	1.9 - 2.2
Observed mean ± SD (n)	3.82 ± 1.69 (28)	3.60 ± 1.94 (29)	2.59 ± 1.62 (28)

Table 3.6 Clutch Sizes Predicted by the Time Limiting Multiple Oviposition Model When Seeds Are Currently Egg Laden and Several Other Females Will Subsequently Lay on the Same Seeds.

Predictions calculated using the equation $\hat{N} = [-Tt + (Tt^2 + ToTt(2N^*-x)/i)^{0.5}] / To$. See Table 2.1 for key to symbols.

Experiment 3.8. Effect of Emergence Weight on Lifespan of Virgin Females.

Lifespan of virgin females was positively correlated with emergence weight and increased by about 5 days for each increase of 1 mg in emergence weight (Fig. 3.12). Predictions of the TLMO model are unaffected by variation in lifespan, because long-term rate of fitness gain, rather than lifetime fitness gain, is maximised using this model.

Experiment 3.9. Effect of Female Age and Previous Oviposition Experience on Clutch Size.

The decline in clutch size at low host encounter rates appears to be a function of female age rather than of clutch number (or number of eggs laid; Fig. 3.13). In other words, the decline is due to a behavioural response to a reduction in the amount of time available for laying (T(t)) or to a physiological constraint determined by the age of the female; it not influenced by previous egg-laying experience. This point is illustrated by comparing Figures 3.13(a) and 3.13(b): lines joining clutches laid by females that completed the same number of clutches were indistinguishable when clutch size was plotted against female age (Figure 3.13(a)), but were separated when plotted against clutch number. However, because of the small sample sizes none of these differences were significant and the results must

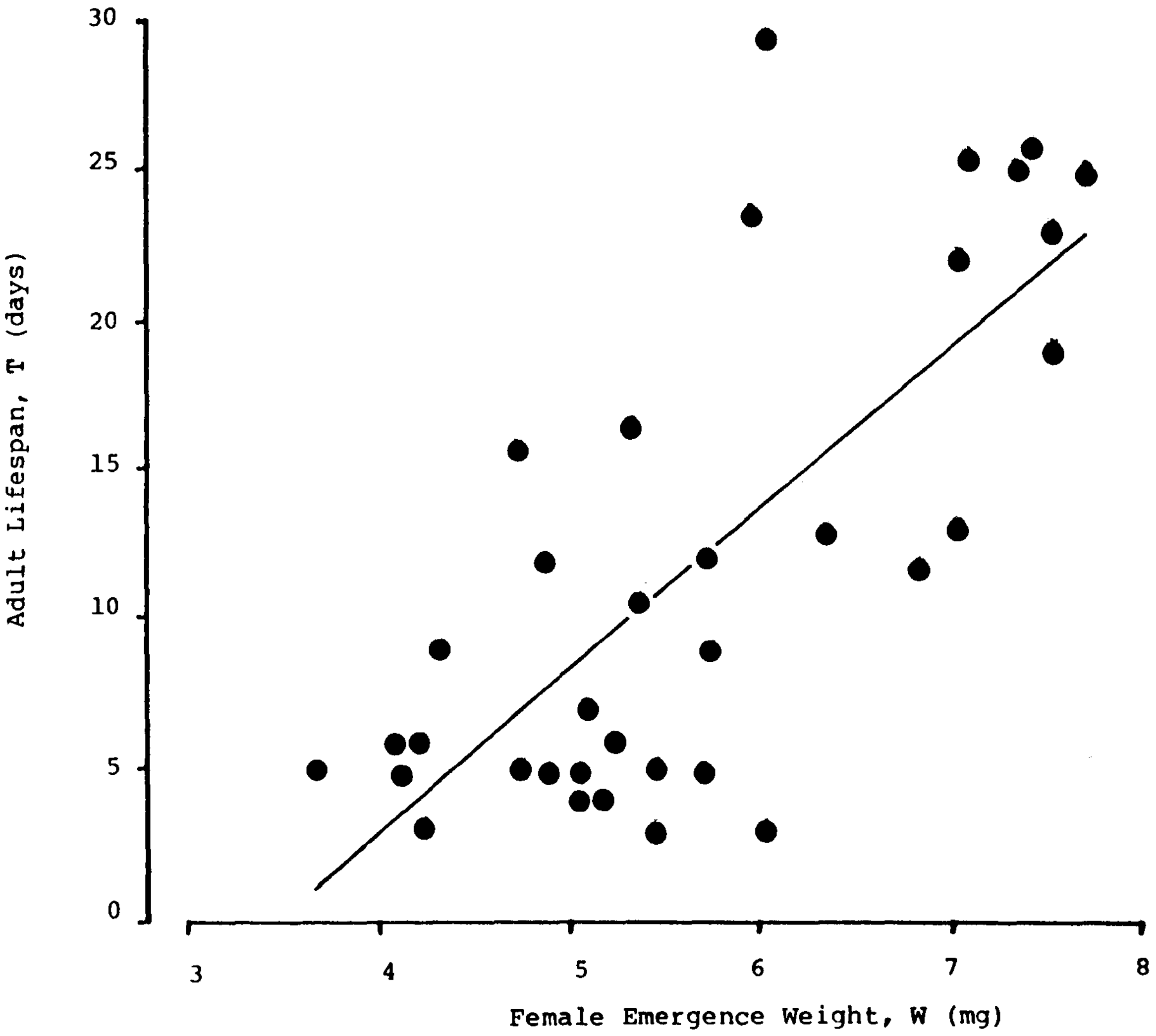


Figure 3.12 Relationship Between Adult Lifespan and Emergence Weight for Virgin Females.

Regression equation: $T = -9.47 + 2.70\ W$; $F = 43.45$, df = 1,33, $P < 0.001$ $r^2 = 0.57$.

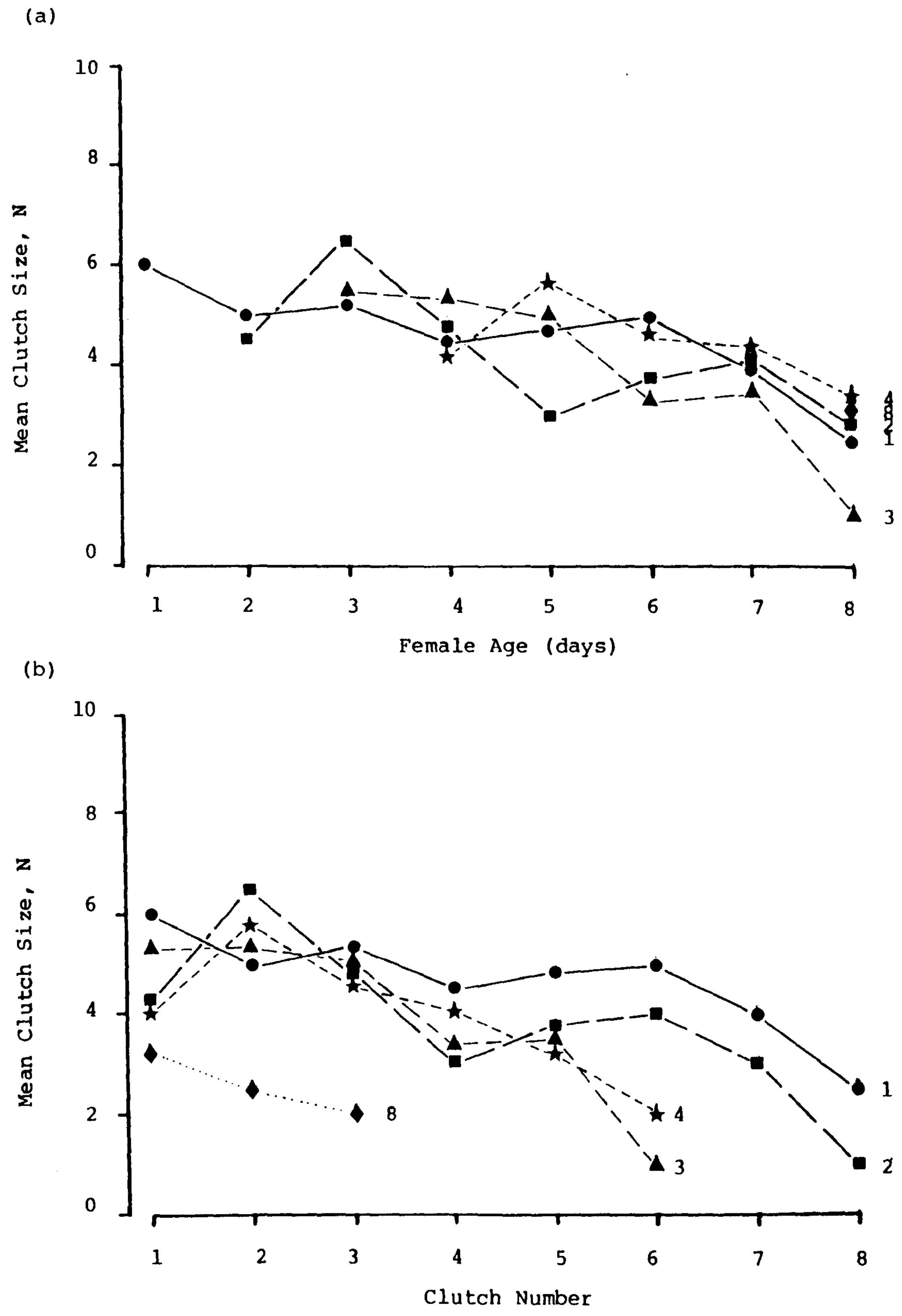


Figure 3.13 Relationship Between Clutch Size and Female Age and Between Clutch Size and Clutch Number for Clutches Laid at Daily Intervals Starting on Day 1, 2, 3, 4 or 8.

Females were given seeds at daily intervals starting on day 1, 2, 3, 4 or 8. Figure (a) plots mean clutch sizes against female age; figure (b) plots the same data against clutch number. Hence, for females given their first seed on day 1, the graphs are identical. Day 1 (●), n=4; Day 2 (■), n=4; Day 3 (▲), n=3; Day 4 (★), n=4; Day 8 (♦), n=4.

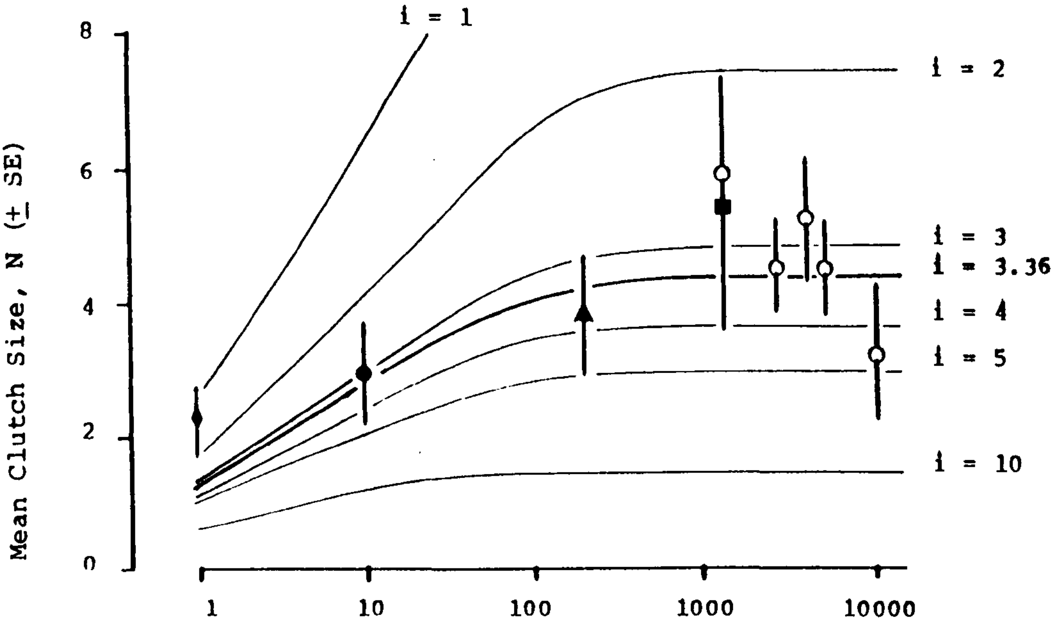
therefore be viewed with some caution.

For females denied seeds for between 5 and 7 days, the duration of seed deprivation did not influence clutch size or the number of mature eggs in the oviducts, and so data for the three days have been combined in the following analyses. Twenty-one females, out of 24, started to lay a second clutch within a minute of finishing their first; and of these females, 19 had mature eggs in their oviducts when dissected immediately after completing the second clutch (mean number of eggs ± SD = 5.25 ± 4.35).

These results strongly suggest that the decline in clutch size after day 5, which is not predicted by the TLMO model, is due to a behavioural response to the reduction in residual lifespan (or residual egg-complement) rather than to egg limitation or physical exhaustion. Moreover, as the maximum clutch size never exceeded about 6 eggs/seed (Fig. 3.13), this suggests that 6 eggs/seed probably corresponds to the most productive clutch size (given, perhaps, that several other females are also likely to oviposit on the same seeds).

When the clutches laid during the present experiment were added to those laid during Experiment 3.5, the fit of the TLMO model decreased only slightly ($r^2 = 0.87$ and 0.86 for the linear and exponential fitness functions, respectively) and the estimated values of i did not differ significantly from those estimated earlier ($t < 0.673$, df = 136, NS; Fig. 3.14).

(a) Linear Fitness Function.



(b) Exponential Fitness Function.

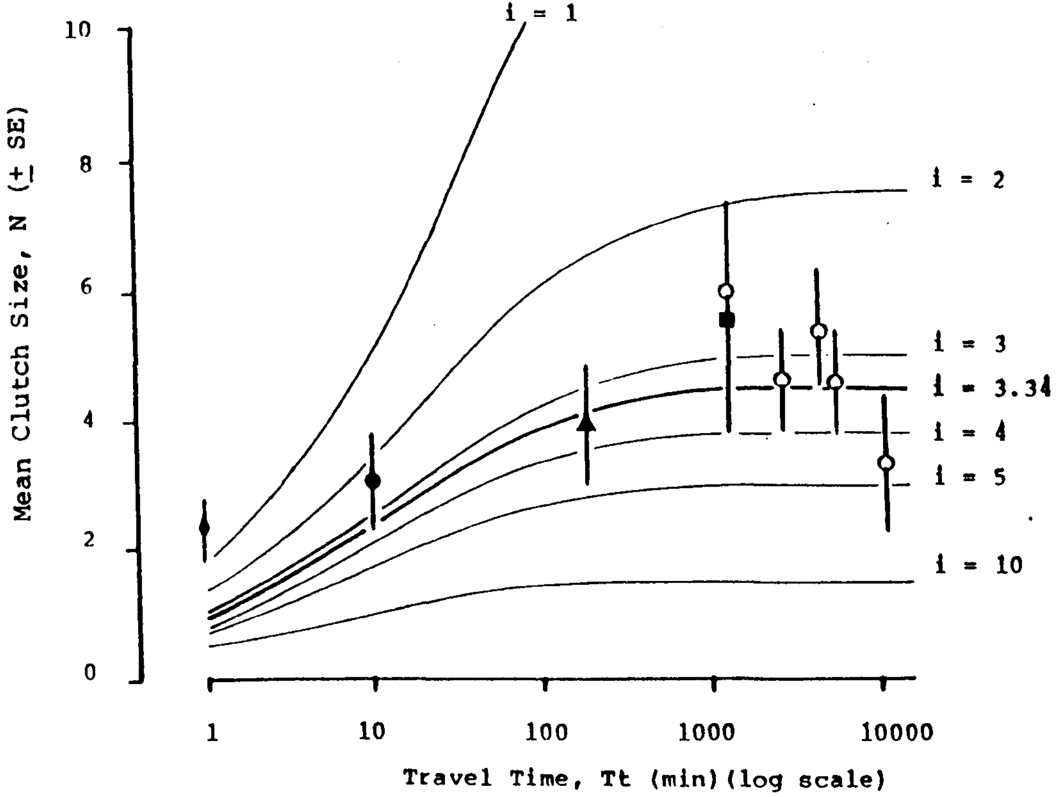


Figure 3.14 Comparison Between Observed Clutch Sizes at Eight Different Travel Times and Those Predicted by the Time Limiting Multiple Oviposition Model with a Linear or Exponential Fitness Function.

See legend to Fig. 3.10. Best fitting model determined by NLLSR:
(a) i=3.36 ± 0.19 (SE); F=468.7, df=1,68, P<0.001; RSS=139.2.
(b) i=3.34 ± 0.19 (SE); F=410.1, df=1,68, P<0.001; RSS=156.0.

DISCUSSION.

In the present study, none of the single oviposition models explained the observed patterns of clutch size with respect to travel time. The hosts-limiting and eggs-limiting models were rejected because observed clutch sizes fell between the values predicted by these two models (**N*** and 1, respectively) and increased with travel time. The reserves, eggs & time and eggs & hosts models were rejected because they predicted clutch sizes of 1 for travel times of 10 minutes or less, whereas observed clutch sizes were between 2 and 3 eggs/seed. The time-limiting model was reasonably accurate at predicting clutch sizes when seeds were encountered at high frequency, but it predicted clutch sizes that were three times larger than those observed when travel times were long (2 hours or longer).

The time-limiting model alone accounted for the observed patterns in clutch size with respect to travel time when multiple oviposition was included as a constraint. At travel times ranging between 1 min and 4 days, and current egg-loads ranging between 0 and 5 eggs/seed, clutch sizes varied in a manner that was in quantitative agreement with predictions of the TLMO model (with i equal to 3-4 females). All of the other models in which clutch size was predicted to increase with travel time predicted clutch sizes that were smaller than those observed at short travel times.

The response to future levels multiple oviposition appears to be fixed; ovipositing females did not adjust their clutch size in response to the presence of conspecific females. This observation adds some (weak) support to the TLMO model and suggests that the number of females encountered prior to oviposition is probably a poor cue to the number of eggs that will subsequently be added to clutches (see Introduction).

Other Explanations for the Observed Trends.

Although the TLMO model explains many of the observed patterns in clutch size, other models may predict the same trends.

Correct currency, incorrect predictions: effect of inaccuracies in measuring total offspring fitness.

Future multiple oviposition must be invoked to explain the observed trends in clutch size because the only model to predict clutch sizes as large as those observed at short travel times (the time limiting single oviposition model) also predicts clutch sizes that are larger than observed at long travel times. An alternative explanation is that the predictions of this model have been incorrectly calculated because some important components of fitness have been ignored. In this respect, there are at least two effects that may be important.

The first is the effect of body size on the fitness of male offspring, which may be positively correlated for several reasons. For example, small males may produce fewer or smaller ejaculates than large males; they may not live as long or compete as well as large males; or they may be discriminated against by females (e.g. Partridge & Farquhar 1983, McLain 1985, Juliano 1985, Simmons 1986a, b). Because adult C. maculatus do not usually feed, small males will probably be disadvantaged (because they will have fewer resources available for reproduction and maintenance). If larval competition has a different impact on the fitness of male than of female offspring, **N*** will differ from that calculated using female offspring alone.

The second is the effect of maternal body size on the fitness of offspring. If small females produce eggs that are less fit than those of large females, perhaps because they are smaller than average, take longer to develop or do not survive as well, then this would also reduce **N*** below its current value. Preliminary evidence suggests that although egg size in the first clutch is negatively correlated with body size, in the long-term, egg size is positively correlated with female body size (K. Wilson & G. Ovenden, unpublished data). Therefore the effect of maternal body size on offspring fitness may have a significant effect on the shape of the LCC.

If the above factors, singly or combined, reduced the most productive clutch size to approximately 6 eggs per seed, then there would be no need to invoke multiple oviposition, and the time-limiting single oviposition model would be the best of the models considered. However, as the effects of female body size are small (c.f. Tables 3.1(a) and 3.1(b)), it is unlikely that the effects discussed above will substantially alter clutch size predictions.

Incorrect currency: time and reserves limiting.

Of the six single oviposition models described in Chapter 2, only the time-limiting model predicts clutch sizes as large as those observed at short travel times. However, this model fails because, at long travel times, it predicts clutch sizes that are 2-3 times greater than those observed. One possible explanation for this failiure is that, at long travel times, females behave as if maximising a different currency, such as reserves (eggs and time, dependently). In other words, at high host encounter rates, time is the major limiting resource and travelling between seeds is costly relative to laying; whereas at low host encounter rates, reserves are limiting and egg laying is costly relative to travelling. This would be the case if the cost, in fitness units, of travelling between seeds was a decelerating function of travel time. Such a function is feasible, and would result if, for example, females spent increasing proportions of their time engaged in energetically inexpensive activities, such as 'lounging' (Ch.4), as the interval between seeds increased. However, even if this were the case, an additional factor would still need to be invoked to explain why clutch size never exceeded 6 eggs/seed even when travel times were greater than a day.

Observed Trends not Predicted by the TLMO Model.

Although the TLMO model accounts for the observed trends in clutch size with respect to travel time, there were two important trends that were not predicted by this model. Firstly, clutch size tended to decline over successive clutches, and secondly, the size of first clutches was negatively correlated with female emergence weight.

The TLMO model fails to predict a decline in clutch size over successive clutches because eggs are not limiting and therefore it always pays females to maximise their long-term average rate of egg-laying (Ch.2 and Appendix 1.2). The decline in the number of eggs laid in each clutch therefore implies that either time is not the only limiting resource, or there is another constraint associated with ageing.

Both of the trends described above are predicted by models 5 and 6 of the present study (see also Begon & Parker 1986). A decline in clutch size with age is predicted if residual egg-complement (**E(t)**) declines at a faster rate than residual lifespan (T(t)), so that residual **T/E** declines over time. Optimal clutch size then decreases in a manner illustrated in Figures 2.3 and 2.4. Note that the opposite trend is predicted (i.e. a temporal increase in clutch size) if **T** decreases at a faster rate than **E**.

Models 5 and 6 predict that small females will lay bigger first clutches than large females if the value of **T/E** at emergence is greater for large than small females (see Figs. 2.3 & 2.4; Appendix 1). In other words, initial clutch size will be negatively correlated with body size if the difference in lifespan between large and small females is greater than the difference in their egg-complements. Comparison of the regression coefficients for lifespan on weight and fecundity on weight (Figs. 3.3 & 3.12) suggests that this is indeed the case: the values for **T/E** at emergence for females weighing 5 mg and 8 mg are 149 and 161, respectively, and this may have a profound effect on the optimal size of the first clutches for the two phenotypes. The size of subsequent clutches will depend on the value of **T/E** at that time, and therefore clutch size trends, with respect to body size, are not immediately obvious after the first clutch. Note that for any given value of **T/E** clutch size will be independent of body size. Similar conclusions were reached by Begon & Parker (1986) for the restricted case of eggs and hosts limiting (or, more precisely, when egg reserves are limiting and females suffer mortality between host visits). These authors extended their analysis to include the effect of mortality rate and egg reserves on the size of eggs produced; they concluded that similar trends may be expected in egg size as in clutch size. In this respect, it is interesting to recall earlier results that the size of the first clutch was negatively correlated with female elytra length, but that the average size of eggs over the first few days of oviposition was positively correlated with body size and declined with age (K. Wilson & G. Ovenden, unpublished data).

To summarise, the TLMO model provides the best quantitative fit the the data with respect to **Tt**, but the results are open to alternative interpretation. Moreover, the TLMO model fails to predict the temporal decline in clutch size and the difference in clutch size between large and small females that alternative models predict. The functional basis for the observed trends in clutch size must therefore remain in some doubt.

CHAPTER 4

PROXIMATE CONTROL OF HOST DISCRIMINATION: RULES AND CUES.

Chapter 4. Proximate Control of Host Discrimination: Rules and Cues.

INTRODUCTION.

Functional models of oviposition behaviour ask *why* clutch size varies under differing conditions, and are generally formulated without reference to the proximate mechanisms underlying them. In Chapter 3, the distribution of egg-loads was best described by the Time Limiting Multiple Oviposition Model, suggesting that time is the major limiting resource and that oviposition behaviour is further constrained by the threat of more than one clutch being laid on each host. The optimal policy (*sensu* Cheverton *et al.* 1985) in this situation is, "leave the host when the rate of fitness gain is equal to that for the whole environment of hosts (given that i-1 other clutches will also be laid on each host)". Implicit in this functional model are assumptions about the female's ability to measure time and about her knowledge of the relative value (*sensu* Ch.2) of all hosts in the environment. Most animals are unable to measure time without error (Gibbon & Church 1981) and, regardless of whether information about average host values is learnt or genetically programmed, it is likely to be subject to errors.

The next step in understanding the evolution of oviposition behaviour is to ask how the optimal solutions are arrived at. In other words, to ask what cues are being used and how these are utilised in decision rules .

The process by which the value of a host or food item is determined is known as 'assessment', and involves the use of appropriate 'cues', such as size or weight. The animal uses these cues to distinguish between items that differ in value. The accuracy with which items are distinguished depends on the resolution of the perceptual mechanism; items are distinguished only if their cue values differ by greater than the degree of resolution. It is difficult to measure the sensitivity of the perceptual mechanism directly, because the animal's 'decision rules' may impose further limits on the level of discrimination observed. For example, female bruchids may distinguish between different seed values precisely, but nevertheless may lay the same clutch sizes on seeds differing in value; i.e. they may not discriminate between them. 'Distinguish' thus refers to the perception of differences by females, whilst 'discriminate' refers to their response to those perceived differences.

Cues for Host Value Assessment.

Host value is primarily determined by factors that limit the amount of food available for the developing larvae. In general, these will include the egg-load of the host and its weight. Many studies have demonstrated that insects are capable of discriminating between hosts differing in these respects, but few have identified the specific cues used (see Vinson 1976 for a review).

Cues used in egg-load assessment.

Several studies have shown that *Callosobruchus* beetles disperse their eggs uniformly over the seeds available to them (e.g. Utida 1943, Avidov *et al.* 1965a, Mitchell 1975, Fig. 4.8 this study). Because females revisit seeds, this suggests that they are able to distinguish between seeds that differ in egg-load, and are deterred from laying on egg-laden seeds.

Messina & Renwick (1985b) performed a series of experiments that enabled them to distinguish between the deterrence effects of the physical and chemical cues associated with eggs. When offered a choice, female *C. maculatus* preferred to lay on pristine seeds rather than seeds from which previously oviposited eggs had been removed, suggesting that a chemical deterrent remains even after the physical structure of the egg has been disrupted (see Oshima *et al.* 1973, for a similar response in *C. chinensis*). Messina and Renwick isolated this 'oviposition marker' and demonstrated that the active portion was soluble in methanol and produced only by ovipositing females; seeds exposed to males stimulated oviposition, whilst those exposed to virgin females had no effect on egg laying.

The physical presence of an egg also contributes to its deterrence effect. Using adhesive, Messina and Renwick glued eggs onto the surface of pristine seeds, and found that females laid fewer eggs on these than on egg-free seeds when the two were offered simultaneously. Moreover, females tended to avoid seeds bearing model eggs made of paraffin wax or watercolour. It should be noted, however, that the model eggs were much larger than real eggs and so the response observed by Messina & Renwick may be part of a more general response to discontinuities in the seed surface: bruchids use tactile stimuli when choosing oviposition sites and avoid cracked or rough seed-coats (Nwanze & Horber 1976).

Messina _et_ _al._ (1987) recently studied the importance of various receptor organs in egg dispersion. In one experiment they removed various appendages from _C._ _maculatus_ (antennae, foretarsi and palps, singly or in combination) and measured how well females distributed their eggs. They found that those lacking palps did not distribute their eggs uniformly between seeds, whereas those lacking antennae or foretarsi dispersed their eggs to the same extent as intact females. However, in a second experiment, antennectomised females with intact palps failed to discriminate between seeds soaked in an ether-extract of oviposition marker and seeds soaked in ether. This, Messina _et_ _al_ suggest, indicates that perception of oviposition marker by the antennae results in reduced oviposition, but that the uniform spacing of eggs between seeds is mainly promoted by perception of contact pheromones (or the physical presence of the egg) by the maxillary and labial palps. Because the cues involved in egg-load assessment are well understood they have not been investigated further in the present study.

Cues used in seed weight assessment.

Several studies of foraging animals have shown that they are able to distinguish between food items of varying profitability (energy value/handling time; e.g. Werner & Hall 1974, Krebs et al. 1977, Houston et al. 1980). Profitability is difficult to measure directly, and therefore some foragers estimate it using prey size as a cue (e.g. Barnard & Brown 1981). This cue performs well in most situations but fails when profitability does not increase with prey size (e.g. when handling times increase in proportion to prey size). Seed weight may also be difficult to measure directly and so bruchids may estimate the value of the seed using other cues, such as surface area and curvature.

Avidov et al. (1965b) performed a series of experiments to ascertain whether bruchids have innate preferences for certain species of seed. The results of their experiments provide some insight into the problem of which cues the bruchids are using to assess seed value. In one of their experiments, they examined the effect of surface area on oviposition behaviour by presenting females with glass rods that were of constant diameter (6mm) but varied in length (from 5 to 40mm). Avidov and his coworkers found that as rod length (and hence surface area) increased, the total number of eggs on each rod increased, suggesting that curvature was not important in determining the number of eggs on each host. However, the density of eggs per unit length of rod remained constant, indicating that surface area may be used as a cue by ovipositing females to disciminate between seeds of differing value. It should be noted, however, that because the ends of the glass rods were covered with felt material to prevent females ovipositing on them, exactly the same result would have been obtained if females distinguished between seeds on the basis of weight rather than surface area.

Because surface area and weight generally covary strongly, it is necessary to manipulate one or other of these variables in order to determine unequivocally which of these two cues ovipositing females use in distinguishing seed value.

Decision Rules for Host Value Discrimination.

The marginal value theorem (m.v.t.) (to which *C. maculatus* behaviour appears to conform; Ch.3) predicts that females should leave hosts when the marginal rate of fitness gain is equal to the *mean* for the environment. Because the gain rate of the larval competition curve for *relatively high* value hosts declines at a slower rate than that for *relatively low* value hosts (see Fig. 4.1.), the residence time (and hence clutch size) predicted by the m.v.t. increases as relative host value increases. In other words, the optimal clutch size is greater for relatively heavy or egg-free seeds than for relatively light or egg-laden seeds. This means that the value of the current host *relative* to the mean for the environment may be more important than *absolute* host size or egg-load in the context of oviposition decisions.

Females may be using one of two main types of rule for discriminating between seeds of different value: an 'absolute rule', in which the oviposition rate is determined solely by the value of the current seed, or a 'relative rule', in which the oviposition rate is determined by the the current seed's value relative to that of previous seed(s) encountered.

Both rules can be further categorised according to how finely seeds are discriminated between. For example, a female may oviposit on a seed with a probability that varies continuously with the value of the current seed (fine discrimination), or she may discriminate only between broad categories of seeds, e.g. pristine and egg-laden, or those weighing more or less than some critical weight, laying on seeds in the same value-category with the same probability

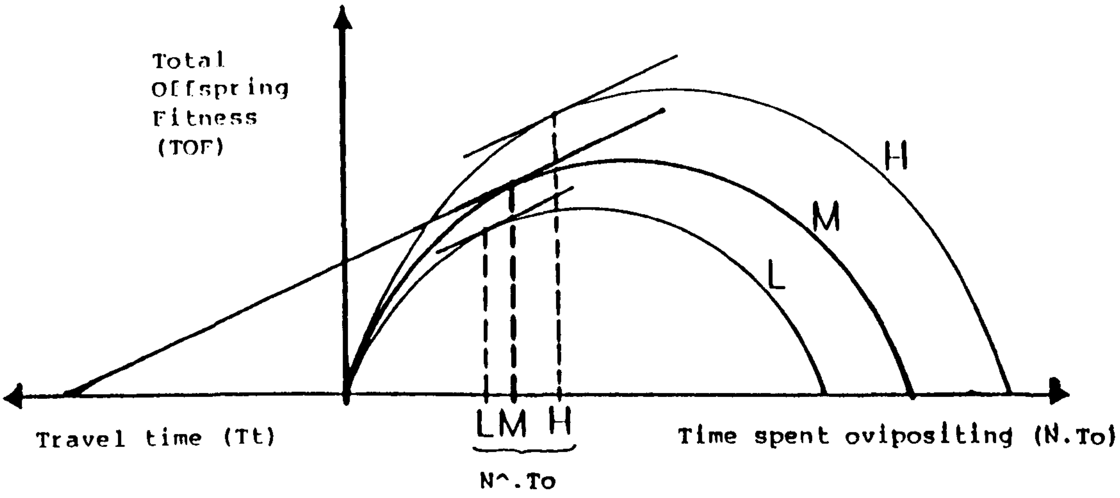


Figure 4.1 Effect of Host Value on Optimal Clutch Sizes.

As host value (curves L, M, and H) increases, optimal clutch size (N^) increases. L = low value host, M = medium value, H = high value.

regardless of their precise value (coarse discrimination).

Egg-load distributions that are more uniform than random can be generated by both relative and absolute rules (Fig. 4.2; Wilson 1988). Moreover, rules in which the probability of laying an egg depends only on whether the seed is pristine or egg-laden, and not on the egg-load of egg-laden seeds (coarse discrimination), also produce more uniform than random distributions at low final egg-loads. However, as expected, they do not function well at higher egg-loads. Exactly analogous distributions can be generated by substituting seed weights for egg-loads.

Distinguishing between absolute and relative rules.

Relative rules of thumb differ from absolute rules in incorporating a mean value for the environment into the decision rule. The two rules may therefore be distinguished by observing how the behaviour of ovipositing females differs in environments of differing mean value. A general scheme for distinguishing between the rules is presented in Table 4.1. At least three different seed values are required (e.g. low, medium, and high), in a minimum of two combinations (out of the three possible; treatments A-C Table 4.1.(a)).

Both types of rule predict that, in all treatments, more eggs will be laid on the higher value seeds (predictions 1-3 in Table 4.1.(b)). Because these

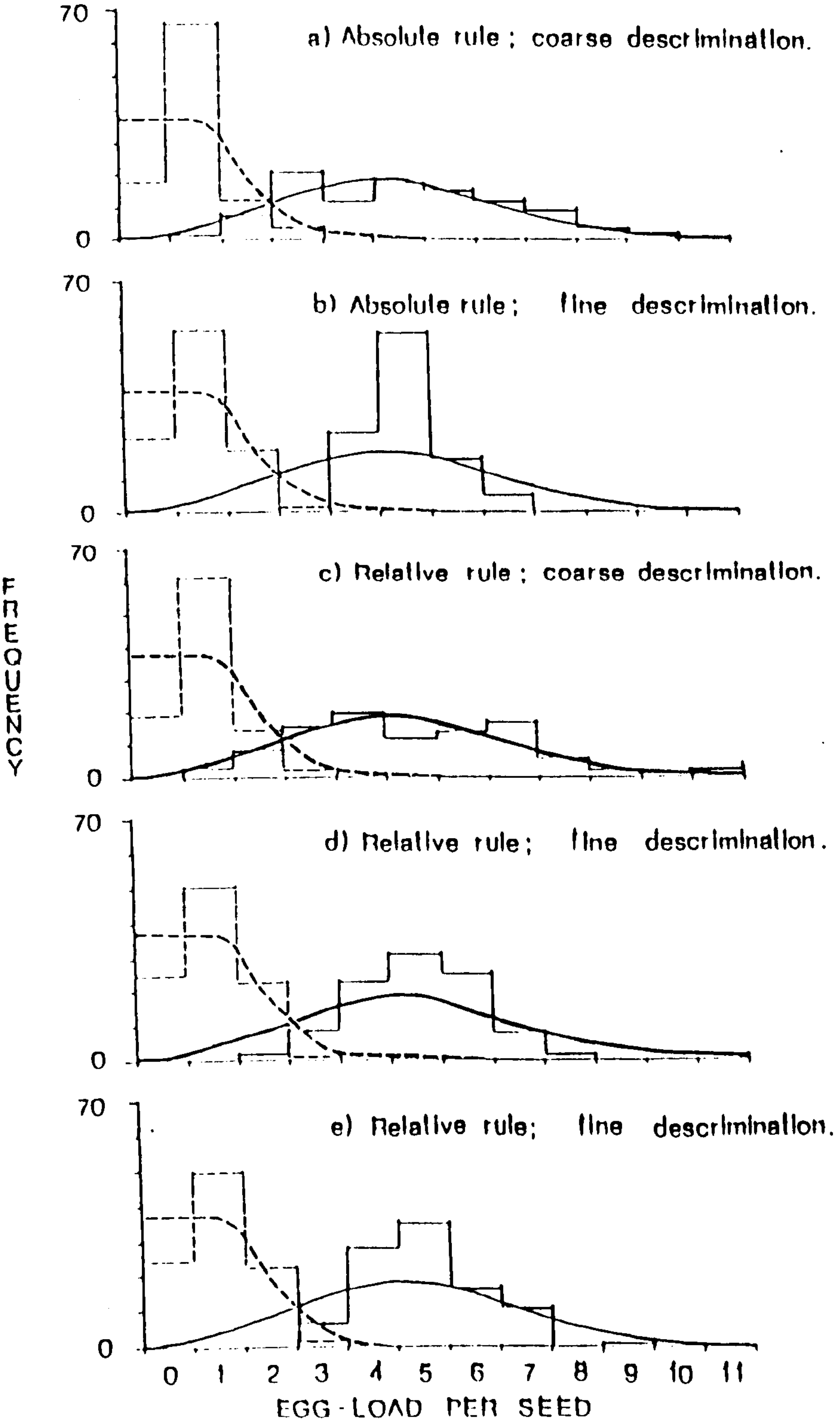


Figure 4.2 Egg Distributions Generated by Computer Simulations of Oviposition Rules

Distributions were generated by Monte Carlo simulations of a single female ovipositing on 100 initially pristine seeds. Females visited (and re-visited) seeds at random, and at each visit laid an egg with a probability that was a function of the egg-load of the current seed, and, in the case of relative rules, of the egg-load of previously visited seeds (see below). Simulations were stopped after either 100 eggs (dashed lines) or 500 eggs (solid lines) had been laid. Fine lines (histograms) are examples of generated distributions; heavy lines (continuous curves) are the expected (Poisson) distributions, when the decision to lay is independent of the current egg-load. All of the predicted distributions, except for the 500-egg distributions in (a) and (c), differ from random (chi-square goodness-of-fit test). The following oviposition rules were used, where p(lay)=the probability of ovipositing on the current seed, c=the current seed's egg-load, m=the mean egg-load at encounter for all seed visits so far, and v=the proportion of seeds that were pristine at encounter for all seed visits so far. (a) If c=0, p(lay)=1. If c 0, p(lay)=0.25. (b) p(lay)=exp(-c). (c) If c=o, p(lay) =exp(-v). If c 0, p(lay)= 0.25exp(-v). (d) p(lay)=exp(-c/(m+1)). (e) If c m, p(lay)= exp(-c/(m+1)). From Wilson (1988).

(a)

TREATMENT	VALUE OF SEED		
	HIGH	MEDIUM	LOW
A	✓ (Ha)	x	✓ (La)
B	✓ (Hb)	✓ (Mb)	x
C	x	✓ (Mc)	✓ (Lc)

(b)

PREDICTION	ABSOLUTE RULE	RELATIVE RULE
1	Ha $\geq$ La	Ha $\geq$ La
2	Hb $\geq$ Mb	Hb $\geq$ Mb
3	Mc $\geq$ Lc	Mc $\geq$ Lc
4	Ha = Hb	Ha $\geq$ Hb
5	Mb = Mc	Mb < Mc
6	La = Lc	La $\leq$ Lc

Table 4.1 Predictions from Absolute and Relative Rules for Three Treatments in which Seed Values Vary.

Seed values H, M, and L refer, respectively, to seeds of high, medium, and low value. Subscripts following seed values refer to treatment. $\geq$ and $\leq$ indicate that the oviposition rate on seeds to the left of the inequality sign will be, respectively, greater than (or equal to) or less than (or equal to) that on seeds to the right of it.

predictions are exactly the same for both rules, they can be used to test the efficacy of the experimental procedure. Failure to detect these predicted differences would cast doubt on any conclusions made from other observations. Clutch size is predicted to change as the mean value of the environment changes _only_ if females are using a fine-discrimination relative rule (predictions 4 & 6). It should be noted that relative rules that involve categorising seeds simply as being above or below a mean value (coarse discrimination) cannot be distinguished from absolute rules using these criteria alone. The only prediction that differs between all absolute rules and all relative rules is prediction 5. This states that when any relative rule is being used, clutch sizes on medium value seeds will be higher when they are paired with low value seeds than when they are paired with high value seeds, but that when any absolute rule is being used clutch sizes on medium value seeds will be the same in both environments. This prediction is therefore the most useful in distinguishing between the two types of rule.

Decision rules for egg-load discrimination.

Mitchell (1975) suggested a decision rule for _C. maculatus_ laying eggs on mung beans (_Phaseolus aureus_), in which females chose between similar-sized egg-laden seeds by comparing the egg-load of the current seed with that of the previous seed. He suggested that they oviposited on the present seed only if it bore fewer eggs than the last seed encountered. This is a somewhat crude relative rule, as the comparison made by the female is between only two seeds. Mitchell produced a simulation of his decision rule which compared favourably with data obtained from ovipositing beetles.

Messina & Renwick (1985a) showed, using choice experiments, that female C. maculatus were able to distinguish between very small differences in egg-load (0 eggs v. 1 egg; 1 egg v. 3 eggs; and 3 eggs v. 5 eggs) and preferentially laid on the lower egg-load seeds. They also demonstrated, by sequentially offering females batches of low (1 egg); intermediate (3 and 4 eggs); or high (5-12 eggs) egg-load seeds, for 1h at a time, that the oviposition rate was strongly influenced by egg-load: females transferred from seeds with low egg-loads to seeds with higher egg-loads decreased their rate of oviposition, whilst the reverse transfer increased it.

These experiments demonstrate that females are not only able to distinguish between egg-laden and pristine seed, but are able to 'count' each seed's egg-load. Messina & Renwick pointed out that the rules governing the decision to oviposit were not clear from their results; ovipositing females may be using a relative or absolute rule. However, as females continued to lay eggs at a low rate for a fúll hour following transfer from low to high egg-load seeds, the authors suggested that if a memory trace of previously encountered seeds is used then it must go back farther than the last seed.

Both Mitchell and Messina & Renwick appreciated that the actual egg-laying rules used by ovipositing females could only be found through closer analysis of searching behaviour during oviposition bouts. In this way, the number of seeds and the time involved in the process of egg-load assessment may be determined.

Decision rules for seed weight discrimination.

Mitchell (1975), using C. maculatus on mung beans, found that egg-load was positively correlated with seed weight, and he incorporated this into his suggested decision rule: "If the present seed is larger than the previous one and bares the same number or fewer eggs then lay an egg on the present seed, otherwise reject it; but, if the present seed is the same size, or smaller than the previous one, then oviposit on the present seed only if it bares fewer eggs than the last encountered". The observed distribution of egg-loads and seed weights was described well by computer simulations of Mitchell's decision rule. However, Mitchell did not suggest a cue by which females could assess seed weight and did not attempt to observe individual oviposition decisions by his animals, arguing that, "oviposition is so infrequent that direct observations are impractical".

Learning Rules.

Insights into the mechanisms involved in behavioural processes, such as oviposition, can often be gained by comparing the observed behaviours of individuals or groups with those predicted by learning rules (e.g. Houston *et al.* 1982, Kacelnik & Krebs 1985, McNamara & Houston 1985). The learning rule model most commonly used by behavioural ecologists is the so-called linear operator model. It is essentially a linear function that expresses allocation of behaviour between behavioural alternatives as a weighted average of past and present experience (Bush & Mosteller 1955). Although this, and most other current learning rules, are essentially just descriptors of behaviour, they are a useful addition to the current debate of the prevalence of learning in oviposition decision rules.

Fitness Consequences of Oviposition Decisions.

Throughout the previous discussion it has been assumed that host value decreases monotonically with increasing egg-load and decreasing host weight. The larval competition curve produced in Chapter 3 (and by several other authors, e.g. Smith & Lessells 1985, Credland *et al.* 1986) suggests that the first part of this assumption is correct, whilst the second part has not previously been examined.

As stated in Chapter 3, there are two methods for examining the consequences of oviposition behaviour (see Perrins & Moss 1975, Hogstedt 1980, Godfray 1987, Pettifor et al. 1988). The observational method (examining the fitness of offspring from naturally produced egg-loads) has been used most frequently, but there are problems associated with it. The first is that females may distinguish between seeds differing in value, and place more eggs on higher value seeds. In this case, egg-loads will be positively correlated with seed value and each individual egg may do equally well on putative 'high' and 'low' value seeds. If females compensate for differences in seed value perfectly, then eggs over seeds will form an 'ideal free distribution' (Fretwell & Lucas 1970) and the fitness consequences of host value will be completely masked. The second problem with the observational method is that females may distinguish between seeds of differing value, and lay similar numbers of eggs on all seeds but place eggs of higher quality on the higher value seeds. This behaviour will tend to accentuate, rather than mask, the fitness consequences of seed value. A solution to the first problem is to manipulate clutch size experimentally, by removing eggs before they hatch. However, there is no easy solution to the second problem.

Both the observational and experimental methods for assessing the consequences of oviposition decisions are employed in the present study. Differences between the two methods in the effect of seed weight on survival can be attributed to the non-random distribution of eggs over seeds.

Aims

The primary aim of the present chapter is to identify the external cues involved in seed value assessment, and how they are used in rules of thumb for oviposition decisions. Experiments focus on the current number of eggs on the seed and seed weight as two correlates of host value. Internal cues involved in the decision-making process are considered in Chapter 6.

Specifically, the following questions are addressed:

(1) Do females distinguish between seeds differing in weight? (Expts. 4.1 & 4.2)

(2) If so, is surface area used as a cue to seed weight? (Expts. 4.3)

(3) Do females distinguish between seeds differing in egg-load? (4.4, 4.5 & 4.6)

(4) Are absolute or relative rules used to discriminate between seeds of differing weight and egg-load? (Expts. 4.4, 4.5 & 4.6)

(4) How well do these decision rules perform in terms of offspring survival? (Expts. 4.7 & 4.8)

MATERIALS & METHODS.

Seed Weight Discrimination.

Experiment 4.1. Discrimination of seed weight by single ovipositing females.

Twenty-nine newly-emerged females were mated and placed separately into 150 ml plastic containers with 10 'small' and 10 'large' seeds. The size of seeds was initially assessed by eye, but those given to nine of the females were also individually weighed on a Sartorius 2462 balance. Seeds categorised as 'small' were always less than 220 mg and those categorised as 'large' greater than 220 mg. For each of the (twenty) females that were not given individually-weighed seeds, the mean weight of 'large' and 'small' seeds was determined. The number of eggs laid on all seeds was determined after 1 day, 2 days, and the death of the female.

Experiment 4.2. Discrimination of seed weight by groups of ovipositing females.

Twenty-five newly-emerged adult females were mated and then left in a 400 ml plastic container with approximately 150 seeds. After about 24 h all females were removed and the weight and egg-load of each seed determined.

Experiment 4.3. Cues used in the discrimination of seed weight by ovipositing females.

The following experiment attempted to distinguish between surface area and seed weight as cues used by ovipositing females in making oviposition decisions.

'Large' and 'small' seeds (defined in Expt. 4.1) were split apart between the cotyledons with a scalpel blade and part of the cotyledons removed using a dentist's drill, care being taken not to damage the seed coat. A 130 mg ball-bearing was placed inside the 'small' seeds, and both seed-types were resealed using wood adhesive. As a result, 'large' seeds had a larger surface area but were of lower weight than 'small' seeds.

Twenty-four newly-emerged, virgin females were each placed in 35 ml containers with a virgin male of similar age and one 'large' and one 'small' seed. Egg-loads on the 'large' and 'small' seeds were determined after each female had died.

The relationship between the surface area and weight of a seed was determined by weighing twenty-five seeds and placing them in tap water for 3-5 min in order to loosen the seed-coats. The testa of each seed was then peeled off and cut so that it would lie flat between two microscope slides bound together with paper-clips. The slide was left for several hours and when the testa was completely dry its outline was copied onto tracing paper. The area of each seed-coat was then determined using a Kontron Electronic GMBH Videoplan digitising tablet.

Decision Rules for Oviposition Behaviour.

Distinguishing between absolute and relative rules.

Oviposition behaviour was examined in two ways. In the first, moment-by-moment decisions made by ovipositing females were observed and recorded in an environment in which seed revisitation was permitted and the encounter rate with hosts differing in value was not controlled. In the second, the encounter rate with seeds differing in value was maintained at a constant level and females were prevented, by removal of the seed, from revisiting hosts.

Experiment 4.4. Are absolute or relative rules used to discriminate between seeds differing in value? I: random encounters with seeds differing in egg-load.

Sixteen seeds were laid out 20mm apart in a 4x4 grid on clean filter paper and presented to mated females for oviposition. Each grid contained seeds of two different egg-loads, and the two 'grid-types' used differed only in mean egg-load. Each row and each column of both grid-types, contained two seeds that bore a single egg. In the 'low-density grid' the other seeds all bore 5 eggs, and in the 'high-density grid' they all bore 11 eggs. As a result, the mean number of eggs per seed on the two grid-types was 3 and 6, respectively (see Table 4.2.(a)) and there were four 'seed-types': 1-egg seeds on low-density grids, 1-egg seeds on high-density grids, 5-egg seeds (on low-density grids) and 11-egg seeds (on high-density grids). Random sampling of the seeds used showed that there was no difference in the mean weights of the four seed-types.

Seeds with suitable egg-loads were obtained by allowing varying numbers of newly-emerged adults (about 16, 80, and 130) to mate and oviposit in 400 ml plastic dishes containing about 100 cowpeas each. After approximately 24 h, adults were removed and the number of eggs on each counted until enough 1-egg, 5-egg and 11-egg seeds had been collected for that day's trials. Thus all eggs were less than 36 h old at the time they were used in the experiment. Because oviposition markers persist after egg shell removal (Messina & Renwick 1985b) the required egg-loads were achieved naturally and not by removing surplus eggs. All females used in tests had not previously laid any eggs, were less than 1 h old when they were placed together with virgin males of similar age, and were left for a further 4 h prior to testing to ensure that they had mated. Preliminary experiments showed that using this method most females would oviposit within the first 20 min of a trial and lay at

(a)

TREATMENT (grid-type)	EGG-LOAD OF SEED		
	1-EGG	3-EGG	5-EGG
High-density (n=16)	✓	x	✓
Low-density (n=16)	✓	✓	x

(b)

SEED-TYPE	ABSOLUTE RULE	RELATIVE RULE
1-egg	H = L	$H \geq L$
5 / 11-egg	$H \leq L$	$H \leq L$
All seeds	$H \leq L$	?

Table 4.2 Predictions from Absolute and Relative Rules for Two Treatments in which Egg-Loads Vary.

H = oviposition rate in high-density grid (mean = 6 eggs/seed);
L = oviposition rate in low-density grid (mean = 3 eggs/seed).
Sample sizes in column 1 of Table (a) refer to Expt. 4.3.

similar rates to each other. Females that failed to lay any eggs in this time probably had not mated and were discarded.

At the start of each trial the female was placed, using a small paint-brush, into the centre of the grid and an inverted 400 ml container placed over the top to prevent escape. For 60 min after the female mounted the first seed, her behaviour was observed and recorded using a BBC microcomputer as a real-time event recorder. Each female was used in only one trial, and there was no significant difference in the mean weights of females on the two grid-types. Trials on the low-density and high-density grids were performed alternately and a maximum of six trials per day was achieved. At the end of each trial, the number of eggs laid was confirmed by counting each seed's egg-load and comparing this with the figures recorded.

Oviposition Behaviour.

Eight mutually exclusive behaviours were defined (Fig. 4.3):

Inspect: time spent walking over a new seed before either stopping (usually prior to oviposition) or leaving the seed.

Still: time spent motionless on the seed surface (usually immediately before or after egg laying).

Laying: time during which the female's ovipositor was

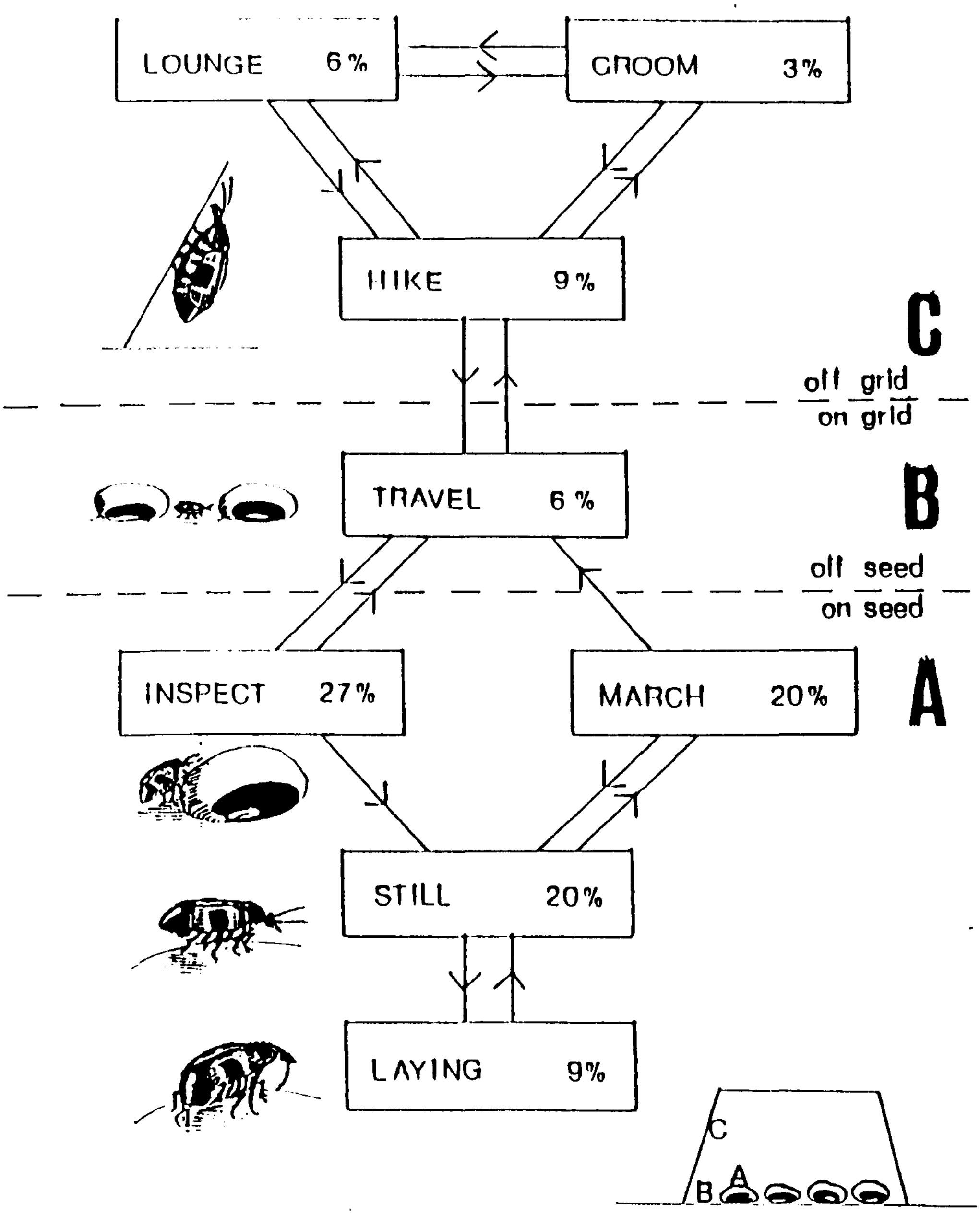


Figure 4.3 An Ethogram for Ovipositing C. maculatus.

Figures given are the mean percentage times spent performing each activity. There were no significant differences in these times between grid-types.

more or less permanently extended.

March: time spent walking over the surface of a seed other than the initial 'inspect' walk.

Travel: time spent on the arena floor between seeds.

Hike: walking outside the boundary of the grid, i.e. on the arena walls.

Lounge: time spent inactive on the sides of the arena.

Groom: time spent cleaning antennae and limbs (usually outside the grid boundary).

'Ovipositition time' was defined as the time spent laying an egg plus any time spent still immediately prior to or following oviposition.

'Acceptance time' was defined as the time taken from arriving on a seed to the start of the still period immediately prior to oviposition.

'Rejection time' was the time spent on the seed in visits that did not result in oviposition.

Using these definitions, egg-laying propensity was measured in the following four ways:

(1) The probability of accepting a seed for oviposition (acceptance probability).

(2) The time taken to accept a seed for oviposition or to reject it (acceptance / rejection times).

(3) The number of eggs laid on each accepted seed.

(4) The number of eggs laid per seed visit.

Each of these four variables was calculated separately for each seed-type and means produced for each female for whom this was possible (a maximum of 16). The values presented in tables 4.8-4.12 are means of those means. Because the data were not always normally distributed, non-parametric tests were used to compare treatments. When rates declined over time, General Linear Modelling (GLM; see Appendix 2) was used to assess the significance of treatment effects (see later). Predictions specific to the present experiment are summarised in Table 4.2(b).

Experiment 4.5. Are absolute or relative rules used to discriminate between seeds differing in value? II: manipulated encounters with seeds differing in egg-load.

The experiment described above has two shortcomings: The first is that it cannot distinguish between an absolute rule and a coarse relative rule in which all egg-loads below the mean are treated the same irrespective of their precise egg-load. The second is that it is extremely time-consuming. Both of these problems are overcome in the following experiment.

Seeds with egg-loads of 1, 3 and 5 were obtained using meehoddddescribed for Experiment 4.4. In order to reduce the variance in clutch size attributable to differences in seed weight, the lightest and heaviest 20% of seeds were excluded from the experiment. Virgin females, aged between 12 and 18 h old, were placed in 35 ml plastic pots with one or more virgin males for approximately 4 h prior to the start of the experiment. Nearly all females of this age will mate within this period (pers. obs.) and approximately 90% of females in the present experiment were observed mating within fifteen minutes of being presented with a male.

Individual females were given seeds of two different egg-loads in an alternating sequence with a 30 minute interval between leaving one seed and being presented with another. At this host encounter rate two conflicting experimental needs are satisfied: to produce enough encounters in the 10 h that the experiment lasted to be able to detect the form of any memory-trace that was being used by ovipositing females, and to produce large clutches, which would increase the probability of detecting any treatment effects. Under the experimental procedure described above, females laid up to eight clutches in 10 h and laid approximately 3-5 eggs in each clutch. Half of the females in each group received a seed of relatively low egg-load first, followed by a relatively high egg-load seed, whilst the other half received seeds in the opposite order. When the female left the seed, the clutch was deemed complete and the seed removed and retained separately. At the end of the experiment, the number of eggs on each seed was counted and females were killed in alcohol, their elytra lengths measured and egg complements determined.

The three treatments used in this experiment are described in Table 4.3(a) and specific predictions

(a)

TREATMENT	EGG-LOAD OF SEED		
	1-EGG	3-EGG	5-EGG
A (n=14)	✓ (1a)	x	✓ (5a)
B (n=15)	✓ (1b)	✓ (3b)	x
C (n=14)	x	✓ (3c)	✓ (5c)

(b)

PREDICTION	ABSOLUTE RULE	RELATIVE RULE
1	$1a \geq 5a$	$1a \geq 5a$
2	$1b \geq 3b$	$1b \geq 3b$
3	$3c \geq 5c$	$3c \geq 5c$
4	$1a = 1b$	$1a \geq 1b$
5	$3b = 3c$	$3b < 3c$
6	$5a = 5c$	$5a \leq 5c$

Table 4.3 Predictions from Absolute and Relative Rules for Three Treatments in which Egg-Loads Vary.

See legend to Table 4.1. Sample sizes in column 1 of Table (a) refer to Expt. 4.5.

summarised in Table 4.3(b). Because clutch size declined over time, differences between treatments were again evaluated using GLM (see later).

Experiment 4.6. Are absolute or relative rules used to discriminate between seeds differing in value? III: manipulated encounters with seeds differing in weight.

The protocol of this experiment was similar to that of Experiment 4.5, except that seeds were egg-free and differed in weight rather than initial egg-load. Seeds were weighed and divided into three non-overlapping categories: 'small' (< 200 mg); 'medium' (> 220 mg, <240 mg) and 'large' (>260 mg). Seeds that fell between these categories were discarded. The mean weights of seeds in each category were 178 mg (n = 153), 230 mg (n = 159) and 293 mg (n = 157), respectively. All seeds were weighed within 48 h following their use. Table 4.4 describes the three treatments used and summarises the predictions of the absolute and relative rule models. Clutch size again declined over time, so GLM was employed once more (see below).

Learning rule simulations.

A simulation model of a simple learning rule was constructed in which the mean value of seeds ($\bar{V}$) was estimated by the arithmetic mean of the current n seed

(a)

TREATMENT	SEED SIZE		
	LARGE	MEDIUM	SMALL
A (n=14)	✓ (La)	x	✓ (Sa)
B (n=13)	✓ (Lb)	✓ (Mb)	x
C (n=15)	x	✓ (Mc)	✓ (Sc)

(b)

PREDICTION	ABSOLUTE RULE	RELATIVE RULE
1	La $\geq$ Sa	La $\geq$ Sa
2	Lb $\geq$ Mb	Lb $\geq$ Mb
3	Mc $\geq$ Sc	Mc $\geq$ Sc
4	La = Lb	La $\geq$ Lb
5	Mb = Mc	Mb < Mc
6	Sa = Sc	Sa $\leq$ Sc

Table 4.4 Predictions from Absolute and Relative Rules for Three Treatments in which Seed Sizes Vary.

See legend to Table 4.1. Sample sizes in column 1 of Table (a) refer to Expt. 4.6.

visits. The learning rule was as follows:

$$\text{If } T < n, \quad \bar{V} = \sum_{i=1}^{T} V_i \; / \; T \qquad [4.1]$$

$$\text{If } T \geq n, \quad \bar{V} = \sum_{i=1}^{n} V_i \; / \; n \qquad [4.2]$$

where T is the total number of seed visits. Note that this rule differs from the linear operator in that, for simplicity, seed values are not weighted according to how long ago they were visited. Whilst this may be unrealistic, the predictions of the models are essentially unchanged by including this complication. In the simulations, alternate seeds were given value rankings of 1 and 3, 1 and 5, or 3 and 5, (the simulations therefore mimicked the experimental protocols of Expts. 4.5 and 4.6, and value rankings were equivalent to egg-load). The value of n ranged between two (the present seed and the previous seed) and eight (the total number of seeds encountered).

Fitness Consquences of Decision Rules.

Experiment 4.7. Variation in fitness with seed weight. I: natural egg-loads.

In Experiment 4.4, twenty-nine females were each offered 10 'large' seeds and 10 'small' seeds. These seeds were subsequently retained separately until all offspring had emerged and the number and sex (but not emergence weight) of all adults to emerge was noted.

Experiment 4.8. Variation in fitness with seed weight. II: manipulated egg-loads.

Manipulated egg-loads of 1, 2, and 4 eggs per seed were produced by allowing mated females to oviposit on pristine cowpeas overnight. The following day, only seeds with more than 4 eggs were retained, and all but 1, 2, or 4 eggs removed with a scalpel blade. Egg-loads were manufactured irrespective of the natural egg-load of the seeds, which ranged from 5 to 15 eggs (mean = 6.05 ± 3.12 SD, n = 144). One week later, all seeds were checked and those in which all eggs had hatched successfully were weighed and retained separately. The number, sex and emergence weight of individuals from each seed was recorded daily during emergence.

The above procedure was repeated separately for seeds bearing 8 hatched eggs. Initial egg-loads ranged between 10 and 17 (mean = 14.06 ± 3.87 SD, n = 50).

Stepwise General Linear Modelling Procedures.

In Experiments 4.4 - 4.6, clutch size declined over time and therefore the mean clutch sizes of different groups could not be compared directly. This problem was overcome using the General Linear Modelling procedure of the SAS statistical package (SAS Institute Inc. 1985). The GLM procedure allows differences between treatments to be detected when the dependent variable (e.g. clutch size) is influenced by one or more additional independent variables, such as time. The analysis was performed in a series of steps in which terms were systematically removed from the initial model until the most appropriate model was reached (see Appendix 2).

RESULTS

Experiment 4.1. Discrimination of Seed Weight by Single Ovipositing Females.

Females given a choice between small and large seeds laid significantly more eggs on the large seeds (Fig. 4.4). This difference was apparent on day 1 and day 2, but was largest after egg-laying was complete. This was because egg-laying rate was higher on the large seeds even after most seeds were egg-laden (shaded histograms in Fig. 4.4).

Not all females discriminated between large and small seeds. The disparity in egg-loads between large and small seeds increased as the mean egg-load increased (Fig. 4.5). Comparison of figures 4.5 and 4.6. suggests that it is only after mean egg-loads exceed 2 or 3 that seed weight begins to have an influence on the number of eggs laid on each seed.

When data from all 9 females for which individual seed weights were known were combined together, there was a significant positive correlation between final egg-load and individual seed weight (Spearman Rank Correlation: $t = 2.526$, $n = 180$, $P < 0.02$). However, because this correlation may be partly due to differences between females, separate correlation coefficients were calculated for each female and a significance level assigned using Fisher's Combined

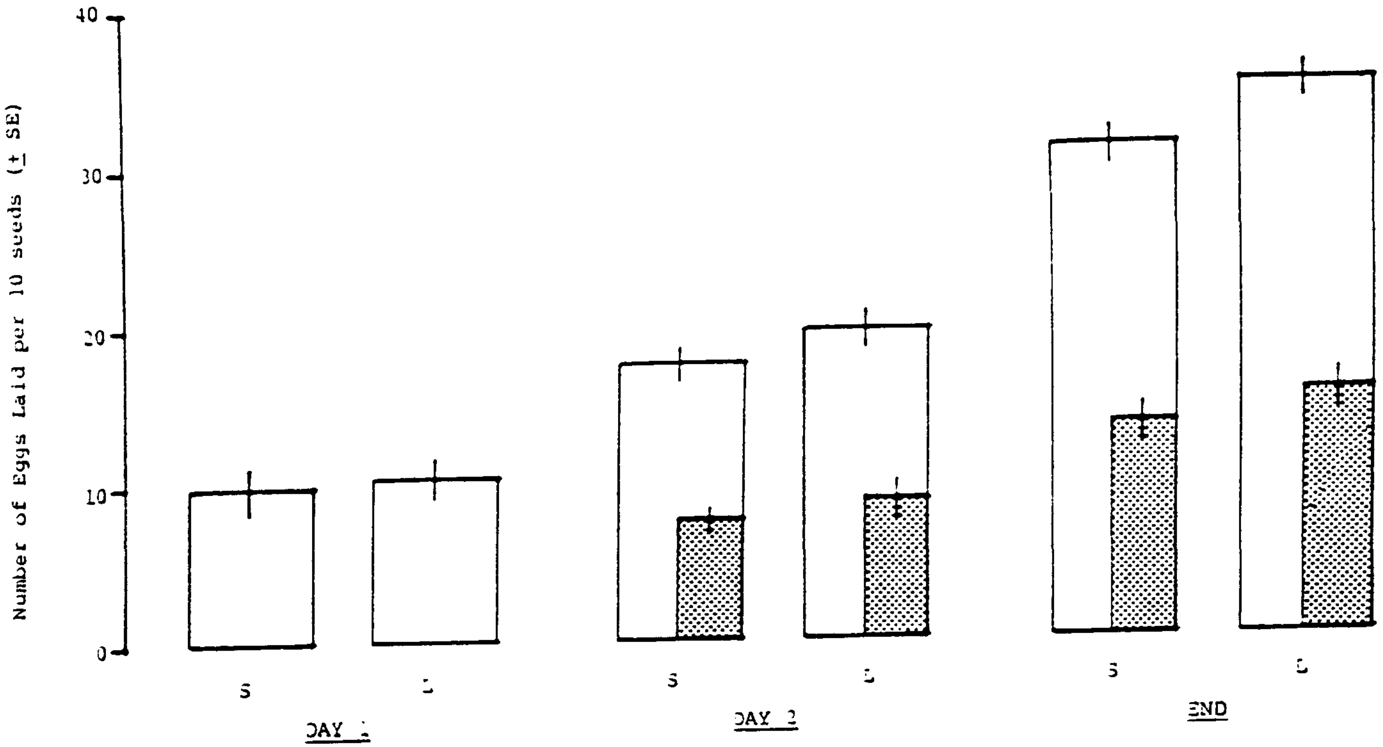


Figure 4.4 Number of Eggs Laid on Small (S) and Large (L) Seeds by Single Ovipositing Females ($n=29$).

Open histograms represent cumulative total number of eggs laid, shaded histograms represent the number of eggs added since the seeds were last sampled.

Wilcoxon T tests (2-tail) for difference between number of eggs laid on large and small seeds:
Day 1: $n=20$, $z=-1.53$, $0.05<P<0.1$; Day 2: $n=28$, $z=-1.89$, $P<0.05$; End: $n=23$, $z=-2.37$, $P<0.01$;
Day 2 - Day 1: $n=27$, $z=-1.32$, $0.05<P<0.1$; End - Day 2: $n=26$, $z=-1.35$, $0.05<P<0.1$.
Note that difference in sample sizes for Wilcoxon tests is due to variation in the number of tied values.

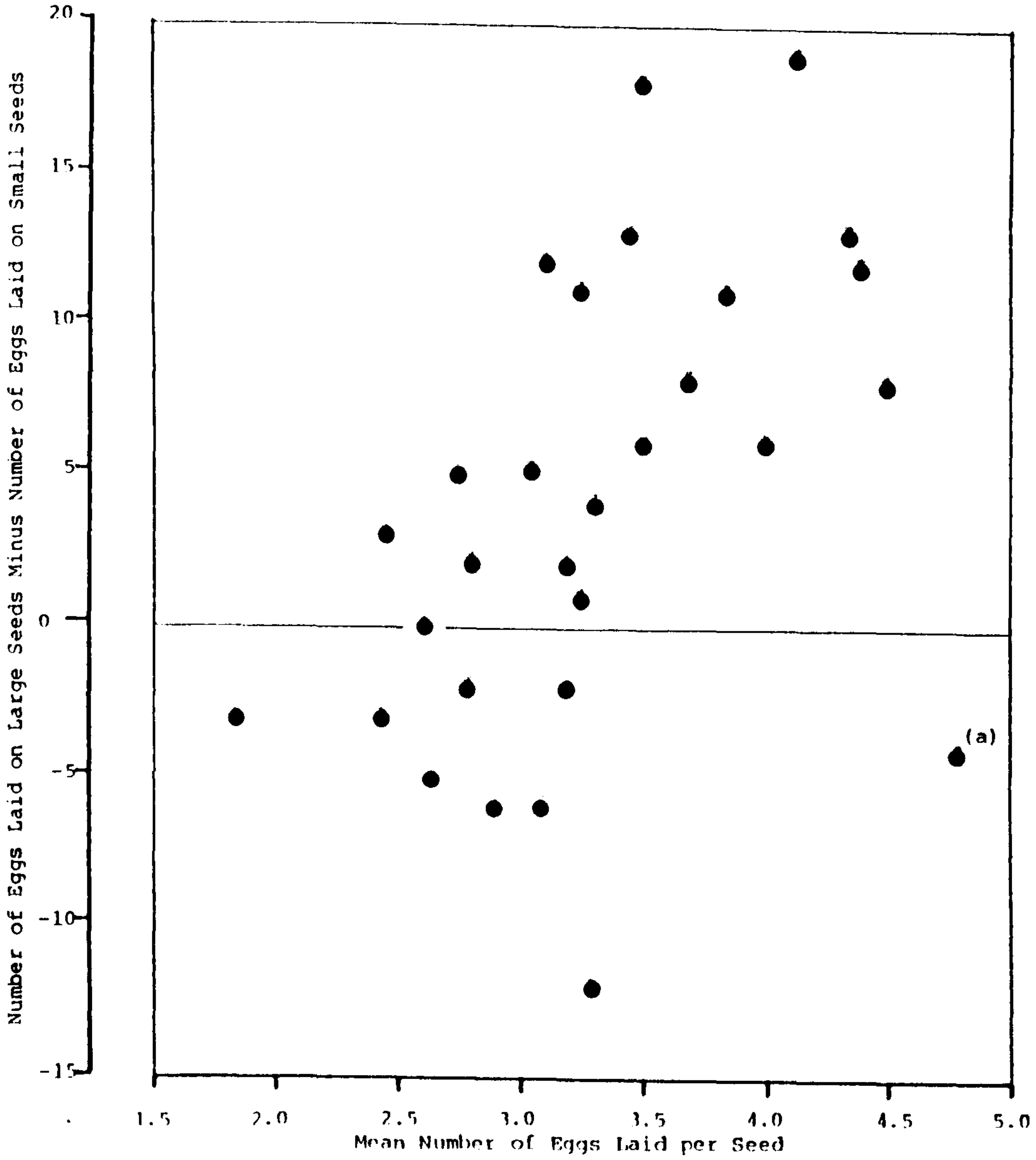


Figure 4.5 Effect of Mean Number of Eggs Laid per Seed on the Disparity Between Number of Eggs Laid on Large and Small Seeds.

Spearman Rank Correlations: All data $r_s = 0.540$, $n=29$, $P<0.01$; excluding point (a) $r_s = 0.612$, $n=28$, $P<0.002$.

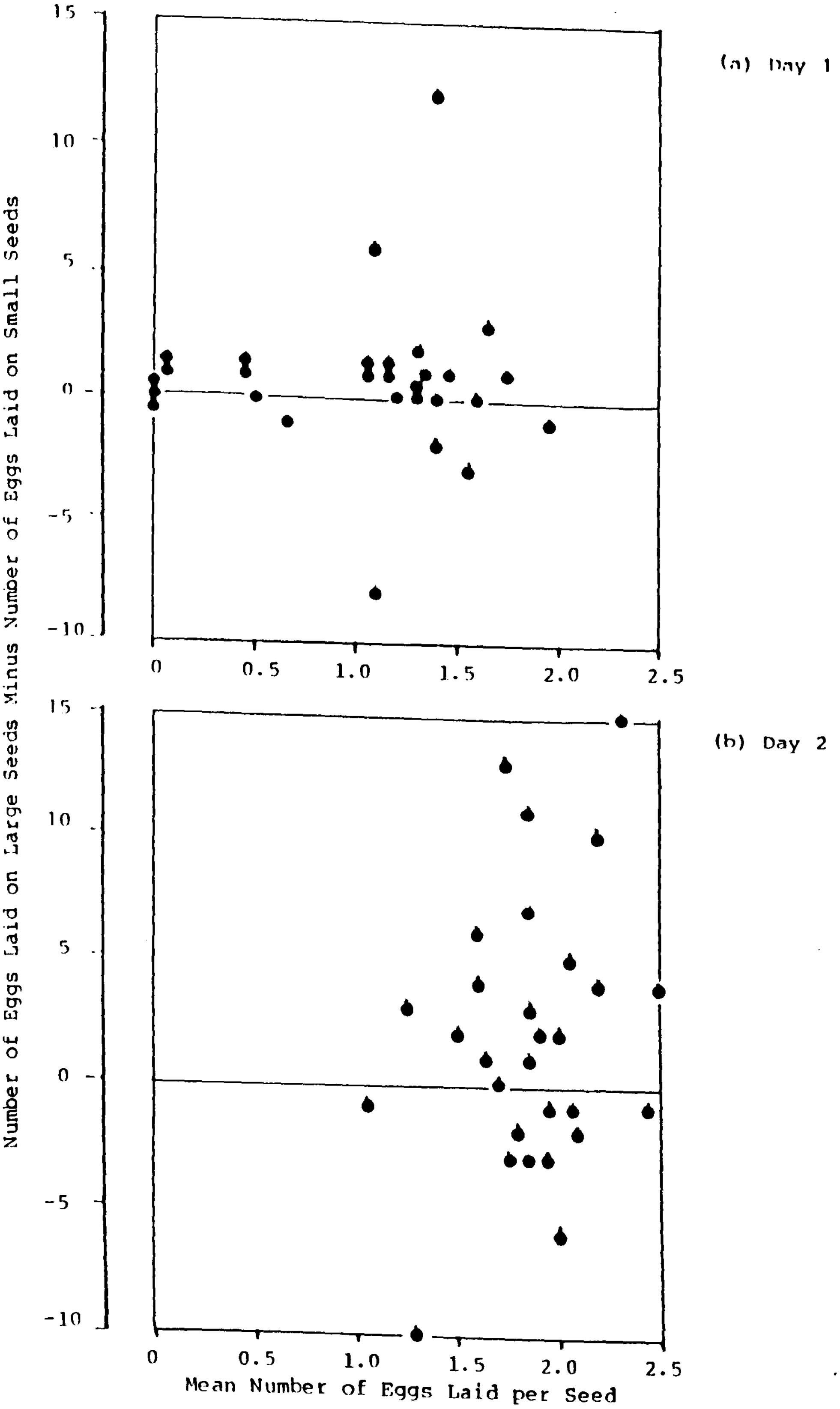


Figure 4.6 Effect of Mean Number of Eggs Laid per Seed on the Disparity Between Number of Eggs Laid on Large and Small Seeds for Day 1 and Day 2.

Spearman Rank Correlations: Day 1: $r_s=-0.011$, n=29, NS
Day 2: $r_s=+0.133$, n=29, NS

Probability Test (Sokal & Rohlf 1981, pp 779-782). Only the correlation between seed weight and final egg-load was significant (Table 4.5), but all other mean and overall correlation coefficients were positive, suggesting that females probably discriminated between seeds from day 1 onwards.

Experiment 4.2. Discrimination of Seed Weight by Groups of Ovipositing Females.

In this experiment, several females were left together to oviposit overnight. Egg-loads ranged between 1 and 9 (mean egg-load = 4.35 ± 1.52 SD) and the variance in egg-loads was significantly less than the mean (variance/mean = 0.53; Fig. 4.7). In other words, eggs were uniformly distributed between seeds. However, egg-load was not correlated with seed weight (Pearson Correlation: t = 0.118, df = 139, NS), suggesting that when several females are ovipositing simultaneously, females discriminate between seeds on the basis of egg-load but not seed weight. This may be because the normal mechanisms for seed weight assessment are disrupted due to interference between females.

DAY	MEAN EGG-LOAD	CORRELATION COEFFICIENT (r_s)			FISHER'S COMBINED PROBABILITY	
		All Seeds (n=180)	RANGE (n=9)	All Females (n=9)	Chi-square (18df)	P
Day 1 (D1)	1.00	+0.068	-0.175 / +0.266	+0.090	6.99	>0.99
Day 2 (D2)	1.85	+0.085	-0.066 / +0.319	+0.088	8.24	>0.95
Final (F)	3.31	+0.186	-0.069 / +0.760	+0.222	31.90	<0.025
D2 - D1	+0.82	+0.016	-0.253 / +0.256	+0.016	0.08	>0.99
F - D2	+1.45	+0.081	-0.313 / +0.081	+0.108	15.14	>0.40

Table 4.5 Spearman Rank Correlation Coefficients Between Seed Weight and Egg-Load.

Fisher's combined probabilities were calculated using the probability values associated with each individual female's correlation coefficient (hence n = 9 and df = 18).

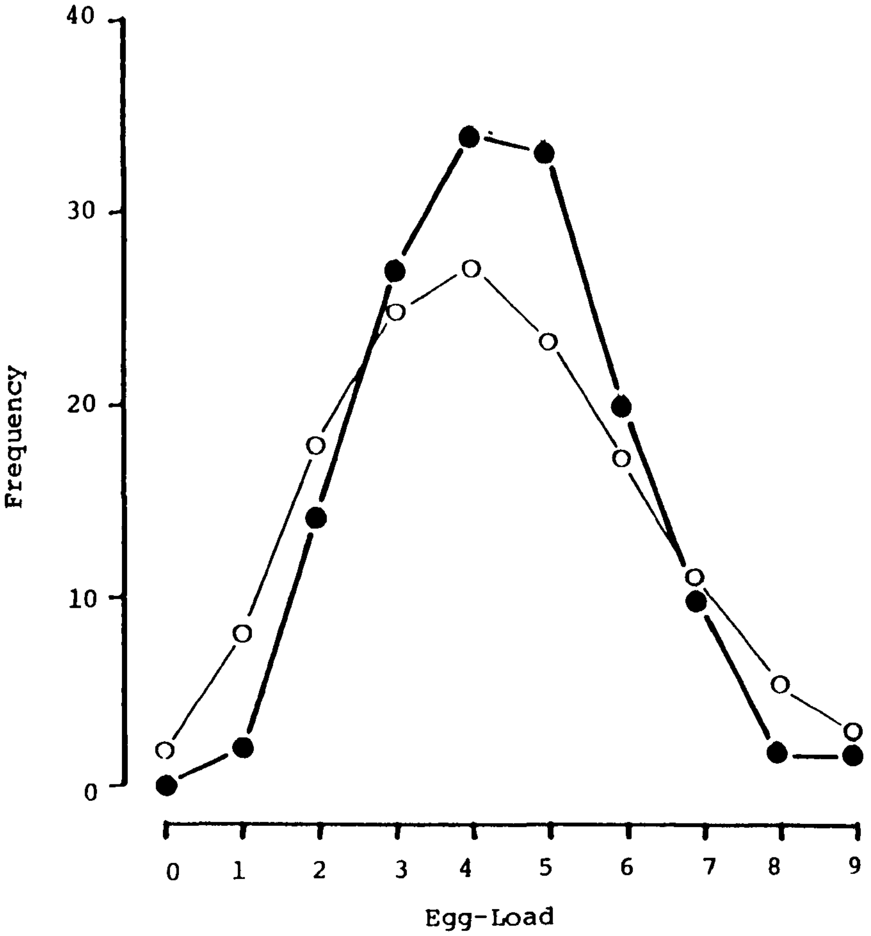


Figure 4.7 Observed and Expected (Poisson) Frequency Distributions of Egg-Loads for Groups of Ovipositing Females (Expt. 4.2).

Goodness of fit: Chi-square = 18.14, df=6, P<0.01.

Experiment 4.3. Cues Used in the Discrimination of Seed Weight by Ovipositing Females.

The surface area of cowpeas was equal to 7.278(seed weight)$^{0.620}$ (Fig. 4.8). The surface areas of seeds used in the choice experiment were estimated using this equation. Manipulated 'large' seeds were significantly lighter than 'small' seeds but had significantly larger surface areas (Table 4.6). Females laid significantly more eggs on 'large' seeds than on the 'small', suggesting that they use the surface area of a seed, rather than its weight, as a cue to its value. The difference in egg-load between large and small seeds was positively correlated with the mean egg-load ($r_s = 0.370$, $n = 24$, $0.1 > P > 0.05$), as in Experiment 4.1.

Experiment 4.4. Are Absolute or Relative Rules Used to Discriminate Between Seeds Differing in Value? I: Random Encounters with Seeds Differing in Egg-load.

Overall oviposition rate.

Females on both high-density (mean = 6 eggs/seed) and low-density (mean = 3 eggs/seed) grid-types laid approximately 11 eggs during the 1 h trial (Fig. 4.9). Egg-laying rate declined over the test period (Fig. 4.10), and the rate of decline did not differ between grid-types

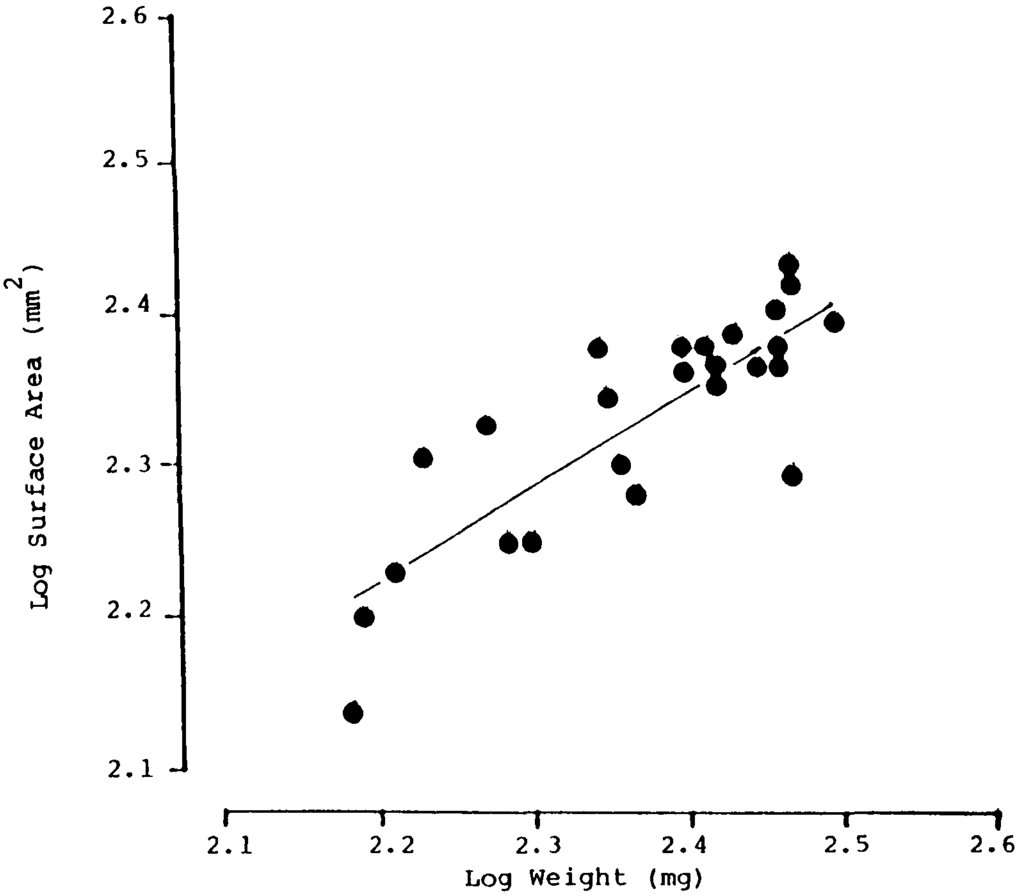


Figure 4.8 Regression of Log Seed Surface Area on Log Weight.

Log surface area = 0.862 + 0.620 (log weight); r^2 = 69%

Surface area = 7.278 $(\text{weight})^{0.620}$

SEED-TYPE	INITIAL SEED WEIGHT (mg ± SD)	FINAL SEED WEIGHT (mg ± SD)	ESTIMATED SURFACE AREA[c] (mm^3 ± SD)	FINAL EGG-LOAD (± SD)
Large (n = 24)	282.2 ± 44.1	211.2 ± 33.0	239.9 ± 24.1	25.0 ± 10.2
Small (n = 24)	213.2 ± 35.5	325.3 ± 35.0	201.6 ± 20.6	21.7 ± 8.3
T[a]	6.418	-20.201	6.456	z = 2.16[b]
P	<0.001	<0.001	<0.001	0.0154

Table 4.6 Surface Areas, Weights and Final Egg-Loads of Manipulated Seeds Used in Experiment 4.3.

(a) Paired t-test (23 df); (b) Wilkoxon signed-ranks test; (c) estimated surface area = 7.278 * (initial seed weight$^{0.620}$), see text.

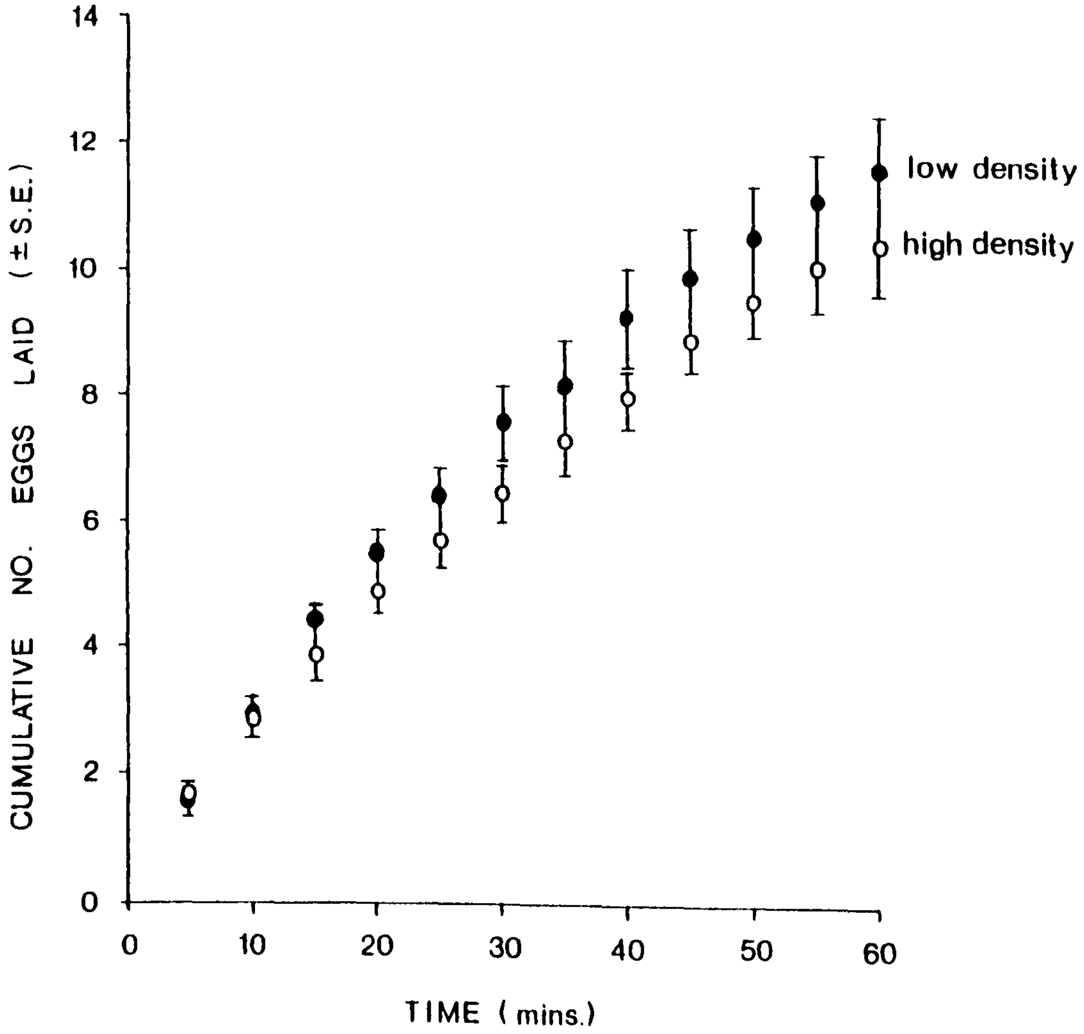


Figure 4.9 Cumulative Number of Eggs Laid on High and Low Egg-Density Grid-Types.

Means calculated using sixteen females for each grid-type.

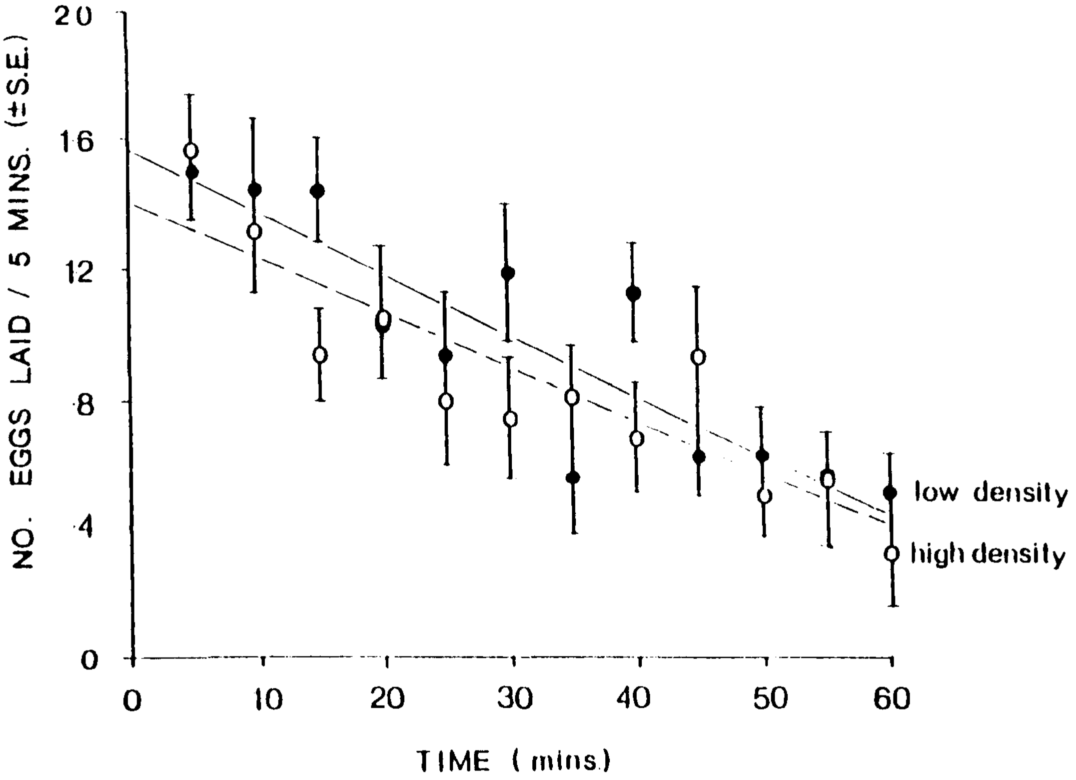


Figure 4.10 Egg Laying Rates on High and Low Egg-Density Grid-Types.

Means calculated using sixteen females for each grid-type. See text for details of analyses.

(GLM: test for significance of interaction terms (slope): $F_{1,380}$ = 0.276, NS; test for significance of treatment term (intercept): $F_{1,381}$ = 1.831, NS). If an absolute rule was being used, a higher overall rate of oviposition would be expected on the low-density grid than on the high (Table 4.2). Although the difference was not significant, the regression line for the low-density grid was slightly higher than that for the high-density grid, therefore an absolute rule cannot be discounted at this stage. No prediction can be made for a female using a relative rule (Table 4.2).

Probability of seed acceptance.

1-egg seeds.

The overall probability of accepting 1-egg seeds for oviposition did not differ between grid-types (Table 4.7). However, because the probability of accepting a 1-egg seed for oviposition declined over time in both grid-types (Fig. 4.11(a)), GLM was also used to test for differences between the two treatments (grid-types). The overall relationship was quadratic and did not differ between grid-types (GLM: test of interaction terms: $F_{2,151}$ = 1.03, NS; test of treatment term: $F_{1,153}$ = 0.02, NS). As the data were not normally distributed, this result was checked using a non-parametric test: none of a series of Fisher Exact Probability Tests, comparing the proportion of females from each grid-type that oviposited during each 10 min period of

SEED EGG-LOAD	HIGH-DENSITY GRID-TYPE		LOW-DENSITY GRID-TYPE
1	0.46 ± 0.07 [16]	U = 103, NS	0.53 ± 0.13 [16]
	T = 0, ***		T = 0, ***
11 / 5	0.02 ± 0.01 [16]	U = 77.5, (*)	0.04 ± 0.01 [16]

Table 4.7 Probability of Accepting a Seed for Oviposition (mean ± SE [n]).

Within grid-type comparisons are made using Wilcoxon matched pairs test; Between grid-type comparisons are made using Mann-Whitney U-test.
Probability values: NS = P>0.1, (*) = P<0.1, * = P<0.05, ** = P<0.01, *** = P<0.001.

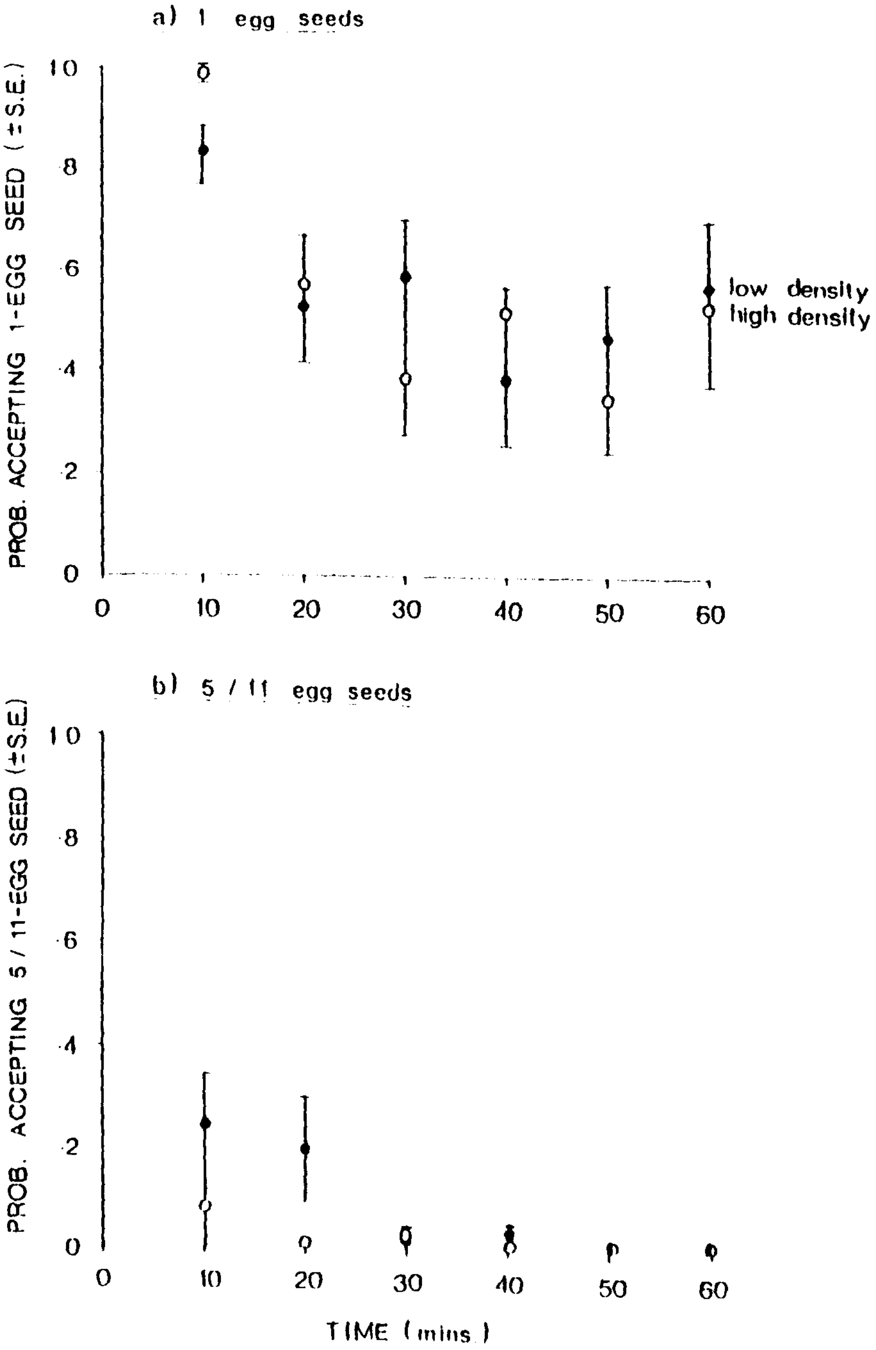


Figure 4.11 Acceptance Probabilities for Seeds on High and Low Egg-Density Grid-Types.

Means calculated using only females that inspected seeds during each 10 min period, therefore sample sizes vary between 8 and 16 for each grid-type. See text for analyses.

the trial, approached significance. It therefore seems safe to conclude that the probability of accepting a 1-egg seed for oviposition was independent of the mean egg-load of the grid. This result is consistent with the predictions of the absolute rule model (Table 4.2).

5-egg / 11-egg seeds.

Females accepted a significantly higher proportion of 5-egg seeds than 11-egg seeds during the 1 h of the trial (Table 4.7). However, the proportion of high egg-load seeds accepted for oviposition declined (linearly) over time (Fig. 4.11(b)) and therefore GLM was used to test for differences between treatments. During the first 20 min of the trial, 5-egg seeds were accepted for oviposition more frequently than 11-egg seeds, but subsequently this difference disappeared. This is reflected in the significance of the interaction term in the model (GLM: test of interaction terms: $F_{1,159} = 7.43$, $P = 0.007$; test of treatment term is therefore inappropriate). This result was checked using a non-parametric test. There was a significant difference in the proportion of females that oviposited on 5- and 11-egg seeds during the first two 10 min periods (Fisher's Exact Probability Test: $P < 0.05$). Thus, there was a significant difference between the probability of accepting 5-egg seeds and 11-egg seeds, as predicted by both models.

Seed acceptance / rejection times.

Acceptance time.

The time between a female arriving at a seed and her laying an egg did not alter significantly during the course of each trial on either grid-type ($r_s \leq 0.192$, $n \geq 79$, NS). Analysis was therefore performed on the mean acceptance times for individuals during each trial. There was no significant difference in the mean acceptance times of 1-egg seeds in the two grid-types. This provides further evidence that an absolute rule may be used by ovipositing females. Females took significantly longer to accept 11-egg seeds for oviposition than 5-egg seeds (Table 4.8). However, it should be noted that only 3 females accepted 11-egg seeds for oviposition (one 11-egg seed per femlale) and that there was no difference in the acceptance times of 1-egg and 5-egg seeds in the low-density grid despite larger sample sizes. The small sample size for 11-egg seeds does not allow the difference between acceptance times on the high-density grid to be tested.

Rejection time.

A comparison of the overall mean rejection times indicates a significant difference between seed-types <u>within</u> each grid-type, but no differences <u>between</u> grid-types (Table 4.9(a)). However, rejection time declined significantly during the trial for all seeds except 1-egg seeds on

SEED EGG-LOAD	HIGH-DENSITY GRID-TYPE		LOW-DENSITY GRID-TYPE
1	67.7 ± 8.1 [16]	U = 120, NS	60.0 ± 3.9 [16]
	(a)		T = 23.5, NS
11 / 5	125.3 ± 23.4 [3]	U = 2, *	71.6 ± 0.01 [16]

Table 4.8 Time (s) to Accept a Seed for Oviposition (mean ± SE [n]).

See legend to Table 4.8. (a) sample sizes too small to perform Wilcoxon test.
Probability values: NS = P>0.1, (*) = P<0.1, * = P<0.05, ** = P<0.01, *** = P<0.001.

(a) Non-Parametric Comparisons of Overall Rejection Times.

SEED EGG-LOAD	HIGH-DENSITY GRID		LOW-DENSITY GRID
1	18.1 ± 4.9 [16]	U=84, NS	22.0 ± 4.4 [16]
	T=23, *		T=22, *
11 / 5	10.5 ± 1.9 [16]	U=100,NS	11.7 ± 1.4 [16]

(b) General Linear Modelling.

EGG-LOADS	INTERACTION TERM(S)			TREATMENT TERM(S)		
COMPARED	F	df	P	F	df	P
1 & 5	2.22	2, 220	NS	1.41	1, 222	NS
1 & 11	0.60	2, 219	NS	8.59	1, 221	**
1L & 1H	3.03	2, 189	(*)	1.41	1, 191	NS
5 & 11	7.13	2, 250	**	0.01	1, 252	NS

(c) Fisher's Combined Probability Tests for a Series of Mann-Whitney U-Tests (see text for explanation).

EGG-LOADS COMPARED	CHI-SQUARE (12 df)	P	No. OF SIGNIFICANT U-TESTS (/6)
1 & 5	4.63	NS	1
1 & 11	23.82	*	2
1L & 1H	8.47	NS	0
5 & 11	13.30	NS	1

Table 4.9 Tests for Differences in the Time Taken to Reject Seeds for Oviposition.

NS = P>0.1, (*) = P<0.1, * = P,0.05, ** = P<0.01, *** = P<0.001

low-density grids ($r_s \leq -0.703$, df $\geq$ 97, $P < 0.05$) and therefore GLM was again used (Table 4.9(b)). There was no significant difference in the time taken to reject 1-egg seeds on the two grid-types ($0.05 < P < 0.1$), or in the time taken to reject 1-egg and 5-egg seeds on the low-density grid. There was, however, a significant difference between the rejection times for 1-egg and 11-egg seeds and between 11-egg and 5-egg seeds (Table 4.9(b)).

Because these data are not normally distributed, the results were checked by performing a series of Mann-Whitney U-tests for the difference between the mean rejection times during each 10 minute period of the test. The overall difference between seed-types was ascertained using Fisher's Combined Probability Test (Table 4.9(c)). These tests indicate that the only comparison that was significant overall was between 1-egg and 11-egg seeds on high-density grids, but for two of the other three combinations there was at least one 10 minute period when there was a significant difference between the seed-types. The comparison of 1-egg seeds between the low- and high-density grid-types produced no significant results.

These three analyses suggest that mean egg-load does not influence the rejection time of 1-egg seeds, that there is a large difference between the rejection times of 1- and 11-egg seeds, and that the time taken to reject 5-egg seeds lies at some point between that of 1-egg and 11-egg seeds.

Number of eggs laid per accepted seed.

The mean number of eggs laid on accepted seeds did not alter significantly during the course of the trial for either grid-type ($r_s \geq -0.193$, $n \geq 79$, NS). The means for individual beetles in the two grid-types were therefore compared when sample sizes allowed. The number of 11-egg seeds laid on was too small for comparisons to be made within high-density grids. None of the other comparisons were significant (Table 4.10).

Mean number of eggs laid per seed-visit.

The mean number of eggs laid per seed-visit is the product of the probability of seed acceptance and the number of eggs laid per accepted seed. There was no significant difference between the mean number of eggs laid on each 1-egg seed encountered on high- and low-density grids. However, significantly more eggs were laid on 5-egg seeds than on 11-egg seeds, and more on 1-egg seeds than on seeds with higher egg-loads (Table 4.11(a)). This provides further evidence that females are using an absolute rather than a relative rule.

In summary, the probability of accepting a 1-egg seed for oviposition, the time taken to accept and reject a 1-egg seed, and the number of eggs laid on accepted 1-egg seeds, did not differ between grids differing in mean egg-load. The

SEED EGG-LOAD	HIGH-DENSITY GRID-TYPE		LOW-DENSITY GRID-TYPE
1	1.71 ± 0.16 [16]	U = 105, NS	1.51 ± 0.16 [16]
	(a)		T = 7, NS
11 / 5	1.33 ± 0.34 [3]	U = 12, NS	1.30 ± 0.24 [16]

Table 4.10 Number of Eggs Laid per Accepted Seed (mean ± SE [n]).

See legend to Table 4.8. (a) sample sizes too small to perform Wilcoxon test.
Probability values: NS = $P>0.1$, (*) = $P<0.1$, * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$.

SEED EGG-LOAD	HIGH-DENSITY GRID-TYPE		LOW-DENSITY GRID-TYPE
1	0.87 ± 0.20 [16]	U = 122, NS	0.84 ± 0.21 [16]
	T = 0, ***		T = 0, ***
11 / 5	0.02 ± 0.01 [16]	U = 79, (*)	0.06 ± 0.02 [16]

Table 4.11 Number of Eggs Laid per Seed-Visit (mean ± SE [n]).

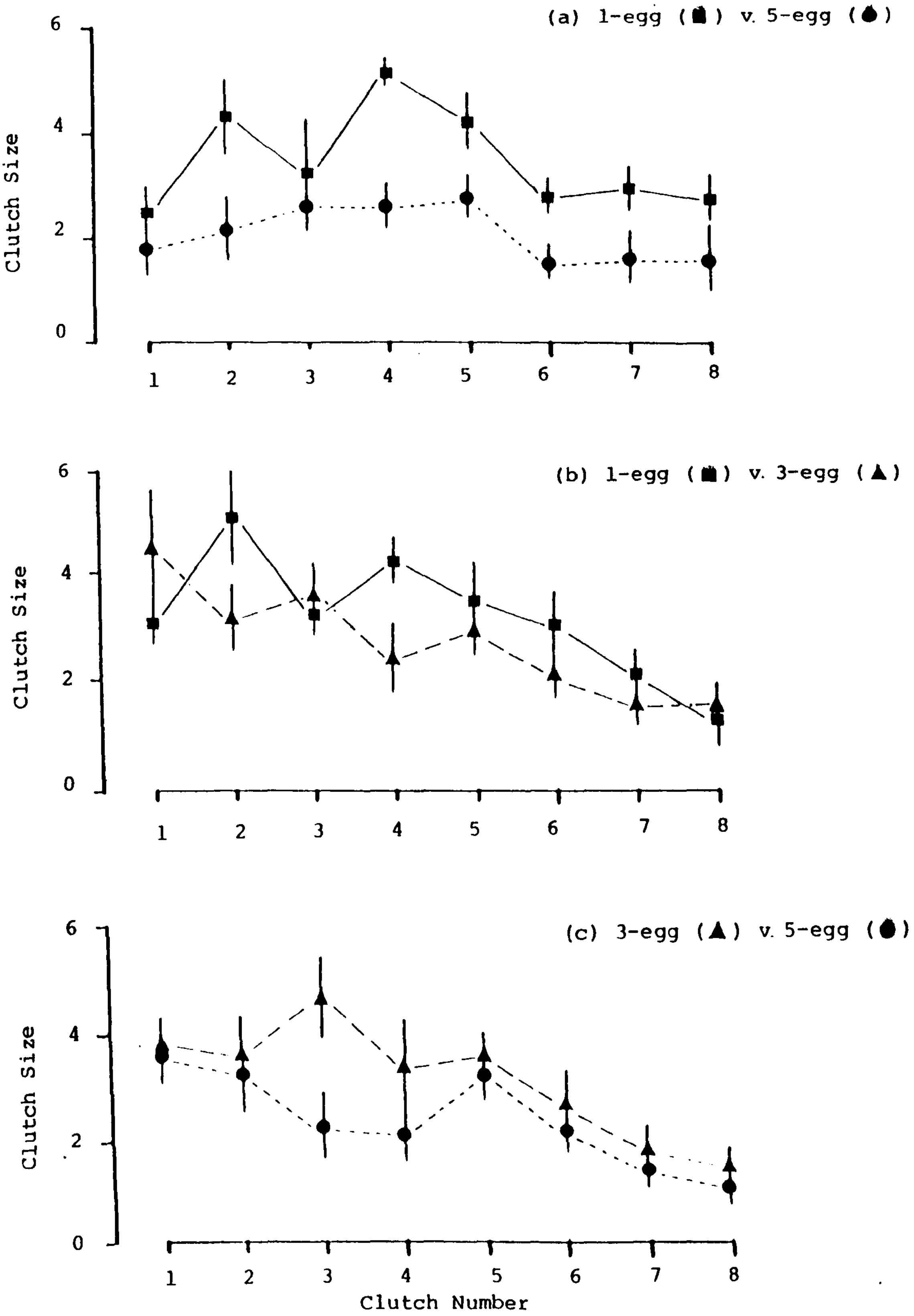
See legend to Table 4.8.
Probability values: NS = P>0.1, (*) = P<0.1, * = P<0.05, ** = P<0.01, *** = P<0.001.

combined effect of the these factors was that a similar number of eggs were laid on 1-egg seeds in both grid-types during the hour-long trial. This result excludes the possibility of a fine-discrimination relative rule being used by ovipositing females, but cannot distinguish between an absolute rule and a crude-discrimination relative rule, in which all seeds with egg-loads below the mean are treated the same.

Experiment 4.5. Are Absolute or Relative Rules Used to Discriminate Between Seeds Differing in Value? II: Manipulated Encounters with Seeds Differing in Egg-Load.

In this experiment, females were offered relatively high and low egg-load seeds in an alternating sequence at 30 minute intervals. The observed clutch sizes are presented in Figure 4.12. Three trends are immediately apparent from this figure: the first is that clutch size declined over time; the second is that clutch sizes on relatively low egg-load seeds were generally larger than those on relatively high egg-load seeds (tests A-C, Table 4.12), confirming the efficacy of the experimental design; the third is that mean clutch sizes often oscillated with clutch number.

Figure 4.13 compares the pattern of egg-laying on seeds with egg-loads of 1, 3 and 5, in grids where the mean egg-load differed. In all cases, clutch size was independent of the egg-load of the alternate seed, and hence mean


(a) 1-egg (■) v. 5-egg (●)

(b) 1-egg (■) v. 3-egg (▲)

(c) 3-egg (▲) v. 5-egg (●)

Figure 4.12 Clutch Size as a Function of Clutch Number for Females Given an Alternating Sequence of Relatively High and Relatively Low Egg-Load Seeds (Treatments A-C, Expt. 4.5).

Sample sizes for treatments A, B, and C are 14, 15 and 14 females, respectively.

TEST	SEED-TYPES COMPARED(1)	TEST OF INTERACTION TERM(S)			TEST OF TREATMENT TERM		
		F	df	P	F	df	P
A	1a & 5a	0.61	2, 106	NS	29.12	1, 108	< 0.001
B	1b & 3b	2.32	2, 106	NS	3.64	1, 108	0.059
C	3c & 5c	0.21	1, 116	NS	6.41	1, 117	0.013
D	1a & 1b	2.38	2, 108	NS	0.42	1, 108	NS
E	3b & 3c	0.00	1, 112	NS	1.88	1, 113	NS
F	5a & 5c	2.53	1, 112	NS	1.55	1, 113	NS

Table 4.12 Comparisons of Clutch Sizes on Different Egg-Laden Seed-Types Using General Linear Modelling Procedures.

(1) Seed-type refers to the egg-load of the seed and its treatment group, such that seed-type 1b refers to a seed with an egg-load of 1 within treatment B, above (i.e. paired with a 3-egg seed.

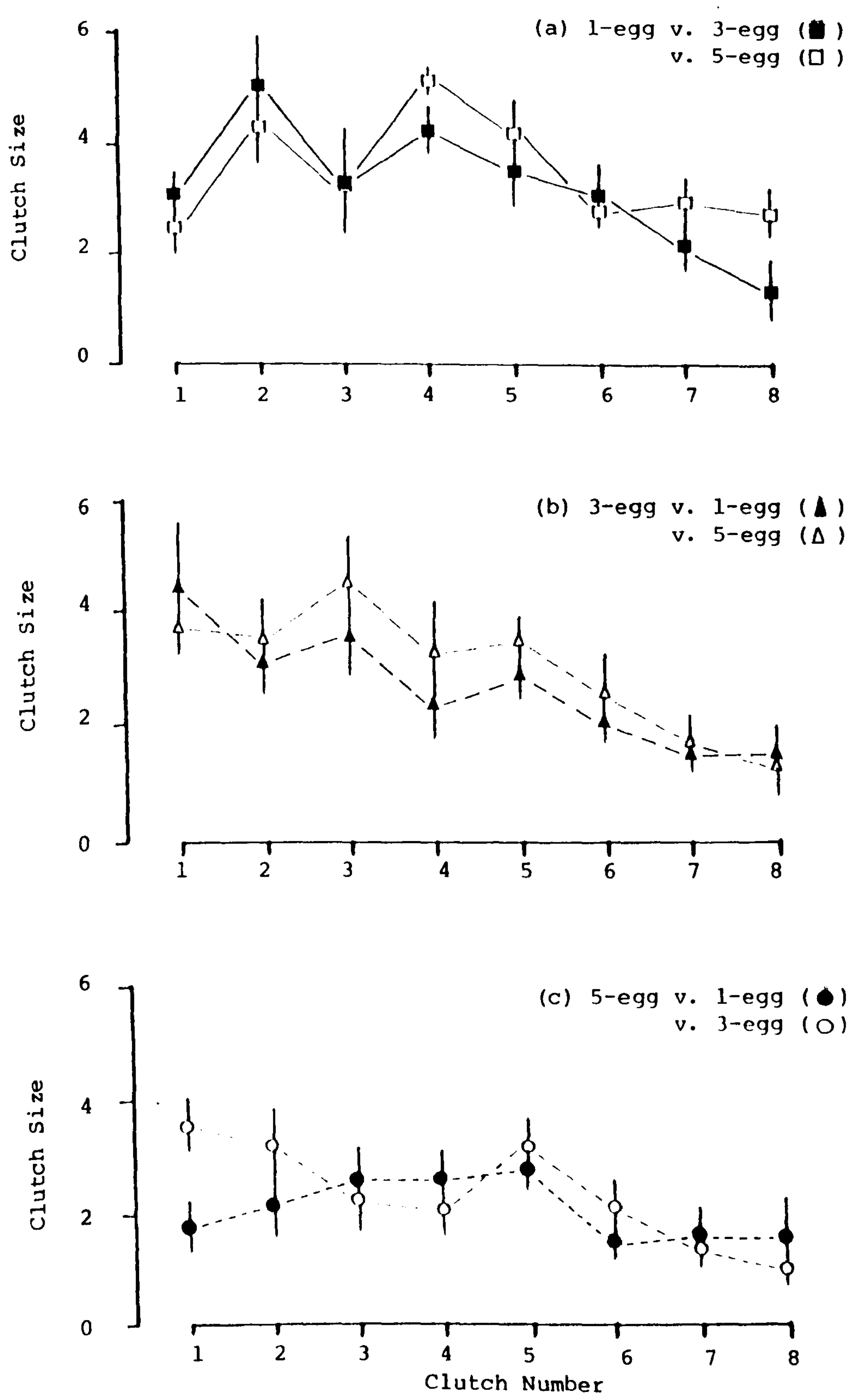


Figure 4.13 Effect of the Egg-Load of the Alternate Seed on the Clutch Size of the Current Seed for Females Given an Alternating Sequnce of Relatively High and Low Egg-Load Seeds (Expt. 4.5).

See Fig. 4.12. Open symbols indicate the lower of the two possible alternate seed types. Note that this data is that in Fig. 4.12 re-plotted.

egg-load (tests D-F, Table 4.12), suggesting that female C. maculatus do indeed use an absolute rule in egg-load assessment (see Table 4.3(b)). The main discriminatory test for the type of rule being used is test E: the comparison of clutch size on 3-egg seeds when the mean egg-load is higher than 3 eggs per seed in one treatment, but lower in the other. The adjusted mean clutch size did not differ significantly between these two treatments (GLM: test of treatment term: $F_{1,113} = 1.88$, $P = 0.173$), and the combined probability from a series of Mann-Whitney U-tests was also non-significant (Fisher's Combined Probability Test: Chi-square = 7.231, df = 16, $P > 0.95$). This suggests that the main determinant of clutch size is the absolute egg-load of the current seed.

Experiment 4.6. Are Absolute or Relative Rules Used to Discriminate Between Seeds Differing in Value? III: Manipulated Encounters with Seeds Differing in Weight.

Females in this experiment were presented with relatively small and large seeds in an alternating sequence at 30 minute intervals. Clutch size in all three treatments declined over time (Fig. 4.14), and more eggs were laid on relatively large seeds in each treatment (though clutch sizes on large and medium seeds in treatment B were not statistically different from each other; test B, Table 4.13). The size of the alternate seed did not affect mean

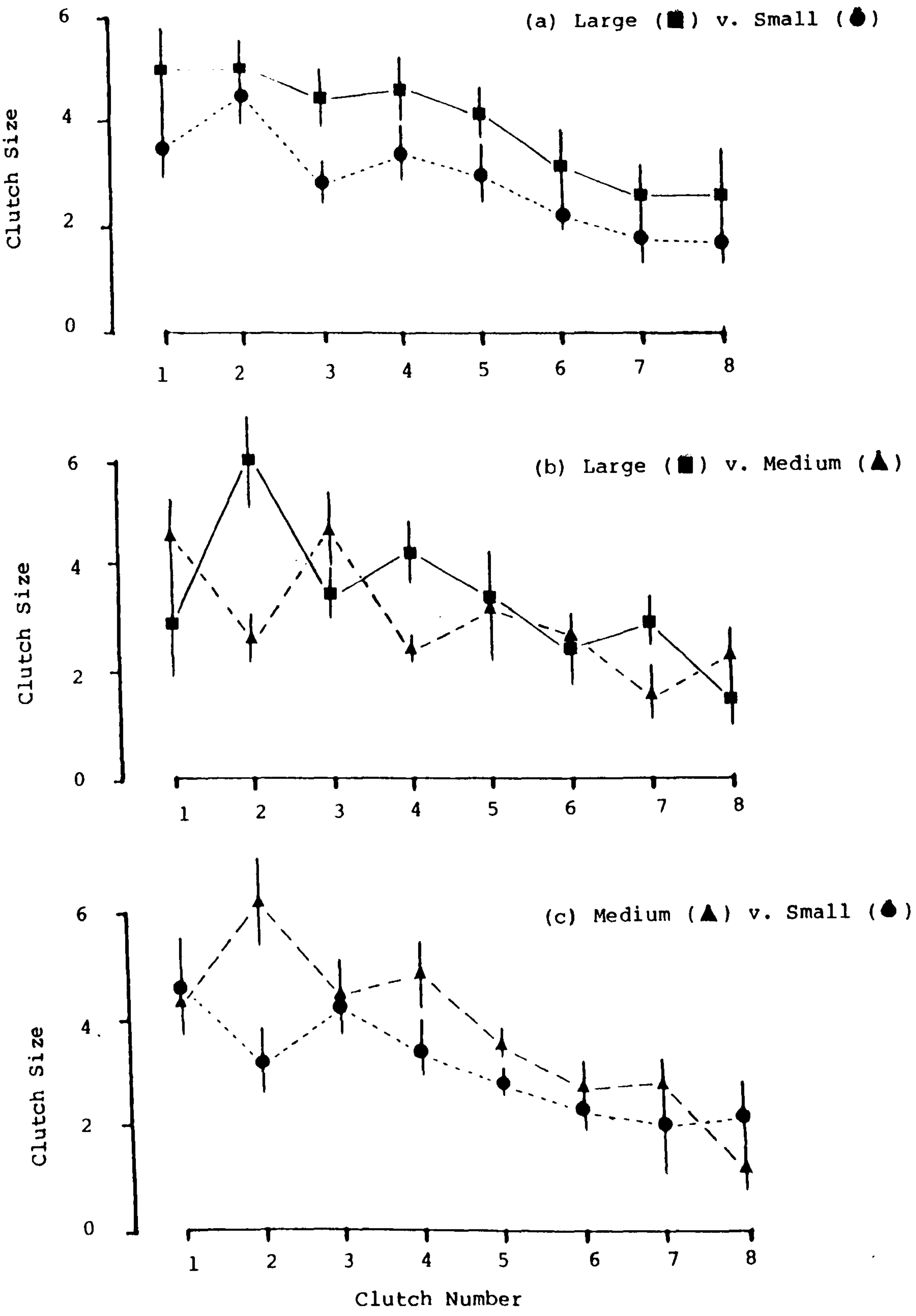


Figure 4.14 Clutch Size as a Function of Clutch Number for Females Given an Alternating Sequence of Relatively Heavy and Relatively Light Seeds (Treatments A-C, Expt. 4.6).

Sample sizes for treatments A, B, and C are 14, 13 and 15 females, respectively.

TEST	SEED-TYPES COMPARED(1)	TEST OF INTERACTION TERM(S)			TEST OF TREATMENT TERM		
		F	df	P	F	df	P
A	La & Sa	1.99	1, 100	NS	5.40	1, 101	0.022
B	Lb & Mb	0.13	1, 116	NS	1.28	1, 117	NS
C	Mc & Sc	0.09	1, 108	NS	14.57	1, 109	< 0.001
D	La & Lb	0.00	1, 112	NS	2.11	1, 113	NS
E	Mb & Mc	1.54	1, 108	NS	4.15	1, 109	0.044
F	Sa & Sc	0.82	1, 104	NS	0.18	1, 105	NS
G	Sa, Sc & Mb [=SS]	0.02	2, 162	NS	0.18	2, 164	NS
H	Mc, La & Lb [=LL]	0.34	2, 162	NS	1.22	2, 164	NS
I	SS & LL	2.80	2, 330	NS	15.75	1, 332	< 0.001

Table 4.13 Comparisons of Clutch Sizes on Different Pristine Seed-Types Using General Linear Modelling Procedures.

(1) Seed-type refers to the weight of the seed and its treatment group, such that seed-type Lb refers to a large seed within treatment B, above (i.e. paired with a seed of medium weight. Seed weights: L = large; M = medium; S = small.

clutch sizes on large and small seeds, but females did lay more eggs on medium seeds when they were paired with small seeds then when they were paired with large seeds (tests D-F, Table 4.13; Fig. 4.15(b)). This suggests that *C. maculatus* uses a crude relative rule to distinguish between seeds by size. Moreover, there was no significant difference between the clutch sizes laid on relatively small seeds in each of the three treatments, or between the relatively large seeds in each treatment (tests G & H, Table 4.13). However, there was a highly significant difference between clutch sizes on relatively small seeds compared with relatively large seeds (test I). This further suggests that absolute seed size is relatively unimportant to oviposition decisions on initially pristine seeds.

Learning Rule Simulations.

The learning rule simulations produced estimates of the current mean value of the environment after arriving at a given seed. The number of seed-visits included in the estimate ranged between 1 and 8. The results of these simulations were as follows (summarised in Table 4.14):

(1) On arrival at the first seed, the estimated mean seed value (and hence clutch size) is independent of the value of the alternate seed (because this is unknown to the female at this time). This result is intuitively obvious and acts as a test for the efficacy of the design of experiments 4.5 and

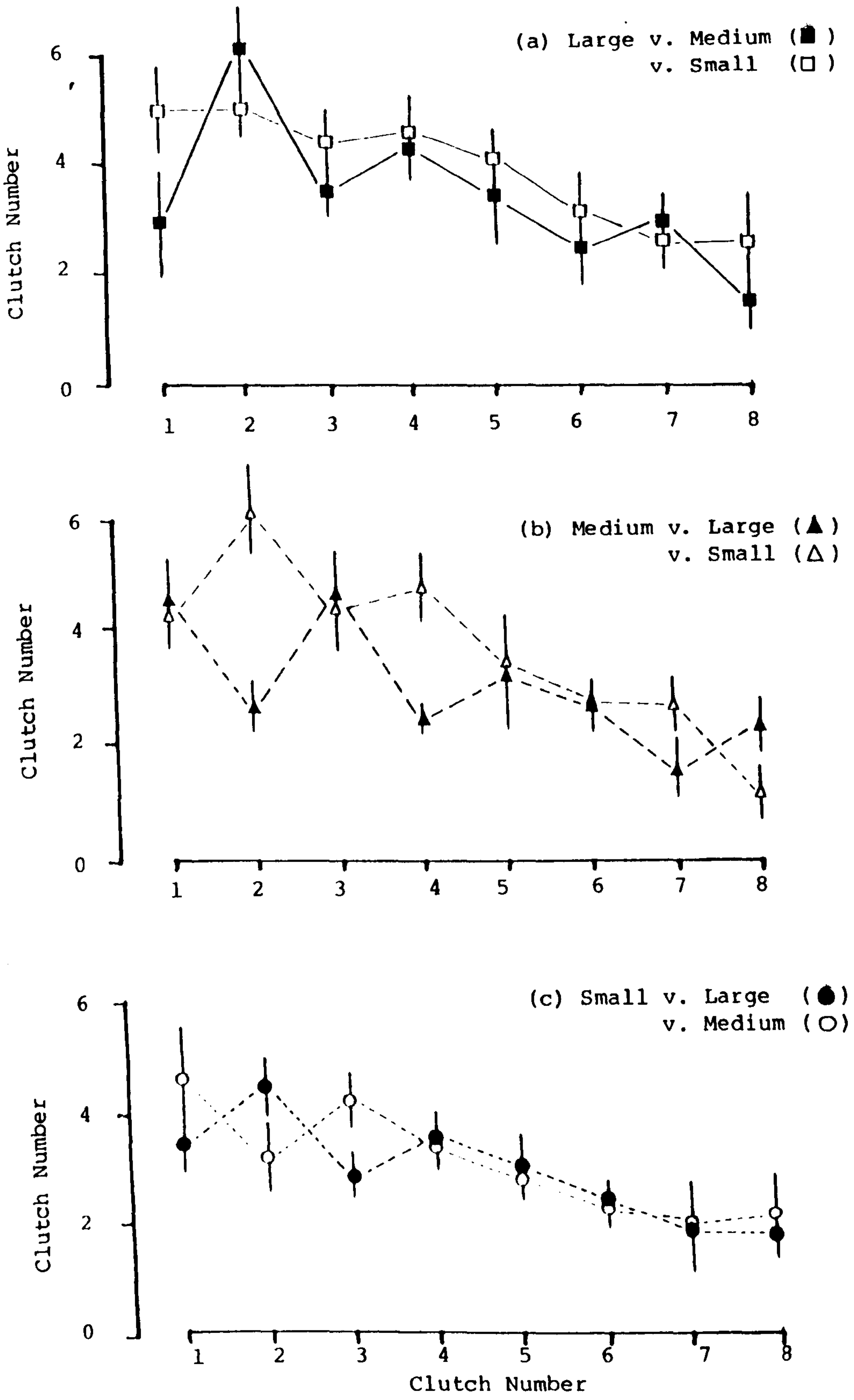


Figure 4.15 Effect of the Size of the Alternate Seed on the Clutch Size of the Current Seed for Females Given an Alternating Sequence of Relatively Heavy and Light Seeds (Expt. 4.6).

See Fig. 4.14. Open symbols indicate the lower value of the two possible alternate seed types. Note that this data is that in Fig. 4.14 re-plotted.

PREDICTED MEAN SEED VALUES	PREDICTED MEAN CLUTCH SIZES
1. At first clutch, estimated mean value is independent the value of the alternate seed.	Size of first clutch is independent of value of the alternate seed (this prediction can therefore be used to test the efficacy Expts. 4.5 & 4.6).
2. Mean value oscillates with respect to clutch number (number of oscillations is equal to the length of memory trace [n, see text]	Clutch size oscillates with respect to clutch number (number of oscillations is equal to length of memory trace [n, see text].
3. Amplitude of the oscillations in mean value decreases over successive clutches.	Amplitude of the oscillations in mean clutch size decreases over successive clutches.
4. Within treatments, mean value on relatively high and low value seeds converge on even-numbered clutches and diverge on odd-numbered clutches.	Within treatments, clutch sizes on relatively high and low value seeds converge on odd-numbered clutches and diverge on even-numbered clutches.
5. At divergence points, mean value on relatively low value seeds decreases and on relatively high value seeds increases.	At divergence points, clutch size on relatively low value seeds decreases and on relatively high value seeds increases.
6. For a seed of given value, mean seed value decreases as the value of the alternate seed decreases.	If a fine-descrimination relative rule is used (see text), clutch size on any given seed will increase as the value of the alternate seed decreases.

Table 4.14 Results of Simulations of Some Simple Learning Rules and Predictions for Clutch Size Variation Based on Such Rules.

See text for description of simulation models used.

4.6.

(2) The estimated mean seed value, on arrival at a seed of a given value, oscillated with respect to clutch number (and hence so will clutch size; Fig. 4.4). The number of these oscillations was equal to the number of seeds included in the calculation of the mean (**n**). This is because the estimated mean was no longer being improved by the addition of an extra seed in the calculation.

(3) The amplitude of these oscillations declined with increasing number of seed-visits as the estimate of the mean seed value converged on the mean of two alternate seed values.

(4) Within treatments, estimates of mean seed value on relatively low and high value seeds converged on even-numbered clutches and diverged on odd-numbered clutches. This was because on even-numbered clutches the estimated mean values for both low and high value seeds were based on an equal number of visits to low and high value seeds, whereas more of one seed value than the other were included in the estimates on odd-numbered clutches.

(5) The oscillations for relatively high value seeds went in the opposite direction (relative to the mean) to those for relatively low value seeds: at the divergence point (odd-numbered clutches), estimated mean values on relatively low value seeds decreased, whilst those on relatively high value seeds increased (Fig. 4.4).

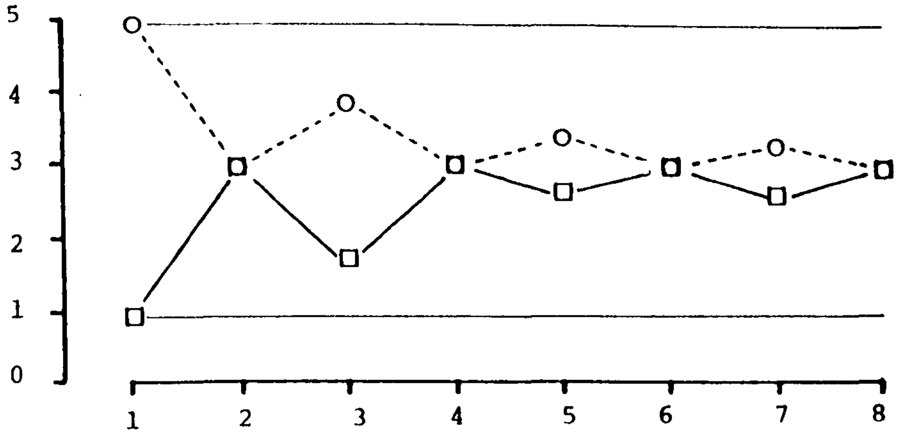
(6) Estimated mean seed value on a given seed increased as

the absolute value of the alternate seed increased.

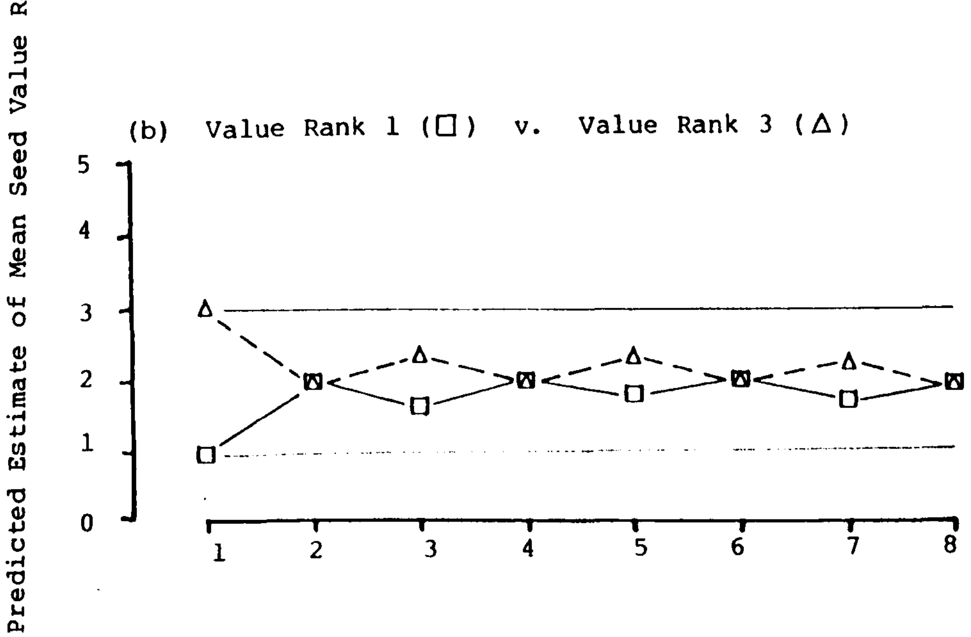
The results of these simulations demonstrate that even a very simple learning rule may have a profound influence on how an animal percieves its environment. The consequences of these learning rules for predicted clutch sizes are summarised in Table 4.14. The most important prediction is that if a learning rule is used by ovipositing females then, under the regime of experiments 4.5 and 4.6, clutch size will tend to oscillate with respect to clutch number (see also Fig. 4.16). The number of these oscillations is determined by the length of the memory window and their amplitude will tend to decrease over successive clutches (as the estimated mean seed value converges on the true mean). Observed temporal variation in clutch size during experiments 4.5 and 4.6 are described well by the learning rule model (Table 4.15).

In Experiment 4.5, five of the six predicted trends in clutch size were supported by treatments A and B (Fig. 4.8(a),(b)), but treatment C more equivocal. A similar pattern was observed in Experiment 4.6. In treatments B and C, five or more of the predictions of the learning rule model were supported, but again the patterns were not upheld by the third treatment (A). In summary, the observed patterns suggest that mean seed value (egg-load and weight) is estimated prior to oviposition using a learning rule similar to the one described above.

(a) Value Rank 1 (□) v. Value Rank 5 (○)



(b) Value Rank 1 (□) v. Value Rank 3 (Δ)



(c) Value Rank 3 (Δ) v. Value Rank 5 (○)

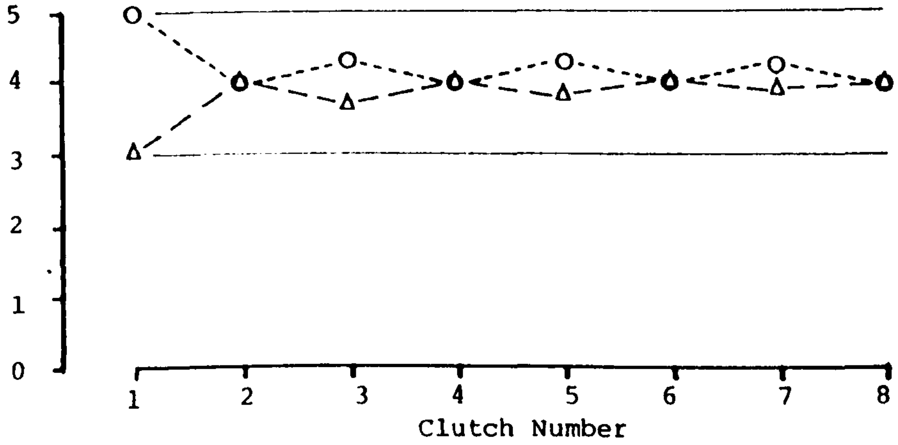


Figure 4.16 Predictions for Estimated Mean Seed Value Rank from a Simple Learning Rule Model for the Case Where the Estimate is Based on an Arithmetic Mean of All Seed Visits So Far (n=8).

Value ranking are such that 1 is of highest rank and 5 lowest (value rank is therefore equivalent to egg-load). See also Tables 4.14 & 4.15 and text.

PREDICTED TREND	OBSERVED IN EXPT. 4.5	OBSERVED IN EXPT. 4.6
1. Size of clutch 1 is independent of the value of the alternate seed.	Yes [Fig. 4.12] ($U \geq 11$, $n1 \geq 6$, $n2 \geq 8$, NS)	Yes [Fig.4.14] ($U \geq 11$, $n1 \geq 5$, $n2 \geq 8$, NS)
2. Clutch size in successive clutches oscillates (number of oscillations equal to minimum length of memory)	Yes [Figs. 4.12(a),(b)] ($\leq$ 5 oscillations per seed-type)	Yes [Figs. 4.14(b),(c)] ($\leq$ 5 oscillations per seed-type)
3. Amplitude of oscillations decrease over successive clutches.	Yes [Figs. 4.12(a),(b)]	Yes [Figs. 4.14(b),(c)]
4. Within treatments, clutch sizes on relatively high and low value converge on odd-numbered clutches and diverge on even-numbered.	Yes [Figs. 4.12(a),(b)] / No [Fig. 4.12(c)]	Yes [Figs. 4.14(b),(c)] / No [Fig. 4.14(a)]
5. At divergence point, clutch sizes on relatively high value seeds increase, and on relatively low value seeds decrease.	Yes [Figs. 4.12(a),(b)] / No [Fig. 4.12(c)]	Yes [Figs. 4.14(b),(c)] / No [Fig. 4.14(a)]
6. Clutch sizes are greater when the alternate seed is of relatively lower value.	No [but see Fig. 4.13(b)]	Yes [Fig. 4.15(b) only]

Table 4.15 Comparison of Observed Trends in Clutch Size and those Predicted by Simple Learning Rules.

The Figures in square brackets refer to examples were the predicted trend is well or badly illustrated.

Experiment 4.7. Fitness Consequences of Oviposition Decisions. I: Natural Egg-Loads.

In Experiment 4.4, females were given 20 seeds: 10 large and 10 small. The present experiment examined the consequences of the decisions made by those females.

When data from all seeds and all females were combined, survival from large seeds (75%, n = 1018) was significantly greater than from small seeds (69%, n = 906; chi-square = 10.38, df = 1, $P < 0.01$). In order to exclude any possible effect due to differences between females, larval survival was calculated separately for each of the 29 females and a mean survival rate on the two seed-types calculated (Table 4.16). Survival was again greater in large seeds than in small (Table 4.16). Therefore, although females compensated for the lower survival rates in small seeds by laying fewer eggs on them (Fig. 4.4), the compensation was not perfect. In other words, they did not produce an 'ideal free' distribution of eggs (Fretwell & Lucas 1970).

Although the overall sex ratio was not significantly different from 0.5, the sex ratio of adults emerging from large and small seeds differed ($P < 0.01$; Table 4.16). Moreover, the adult sex ratio from large seeds was significantly female-biased (one-sample t-test for difference from 0.5: $t = 2.612$, df = 28, $P < 0.02$), whilst that from small seeds was biased towards males, though not

SEED SIZE	OVERALL SURVIVAL (%)		SEX RATIO$^{(a)}$	
Small	69.5		0.531	
		t = 2.631) df = 28 P < 0.02		t = 3.036) df = 28 P < 0.01
Large	75.5		0.462	
All	72.5		0.499	

Table 4.16 Effect of Seed Weight on Overall Survival and Sex Ratio.

Probability values are for paired t-tests on arcsine-transformed data. (a) sex ratio = number of males emerging divided by total number of adults emerging.

significantly so ($t = 1.514$, df = 28, $P > 0.1$).

Table 4.17 presents the correlation coefficients between seed weight and survival for seeds bearing 2-6 eggs, using the 9 females for which all seeds were weighed. This indicates that, when egg-loads were determined by the ovipositing female, survivorship was more or less independent of seed weight, except when egg-loads were high. At 6 eggs per seed the survival rate was positively correlated with seed weight. Interestingly, at the lowest egg-load (2 eggs per seed), overall survival tended to be negatively correlated with seed weight ($P < 0.1$), and as egg-load increased the correlation became increasingly more positive. In summmary, females laid more eggs on large seeds than small, but laid fewer on large seeds than their weights apparently merited. Hence, the survival rate from large seeds remained higher than for small seeds, despite the greater egg-loads on large seeds.

Experiment 4.8. Fitness Consequences of Oviposition Decisions. II: Manipulated Egg-Loads.

Egg-Load.

The effect of egg-load on offspring fitness was examined for seeds with manipulated egg-loads of between 1 and 4 eggs/seed. Egg-loads of 8 eggs/seed were excluded from the analysis because these were not set up at the same time

	EGG-LOAD				
	2 (n=19)	3 (n=38)	4 (n=35)	5 (n=23)	6 (n=14)
Mean seed weight (mg)	168	202	208	203	218
Overall survival (%)	84	73	63	59	62
r_s (survival v seed wt)	-0.46	-0.20	-0.22	+0.19	+0.66
Probability	(*)	NS	NS	NS	**

Table 4.17 Correlations Between Seed Weight and Offspring Survival for Naturally-Produced Egg-Loads Between 2 and 6 Eggs/Seed.

Spearman Rank Correlation coefficients are based on eggs laid by nine females for which individual seed weights were measured (see text). NS not significant; (*) $P<0.1$; ** $P<0.01$

as the lower egg-loads. Analysis was by both correlation analysis (Spearman Rank Correlation test) and non-parametric analysis of variance (Kruskal-Wallis test), the latter being performed in order to increase the possibility of detecting any 'Allee effect' (Allee _et_ _al_. 1949).

Survival declined significantly as egg-load increased ($P < 0.001$; Table 4.18), and adult sex ratio did not change. Male emergence weight declined significantly as a function of egg-load, but at egg-loads of 1-4 eggs/seed female emergence weight was independent of egg-load. The final fitness component considered, the development period from egg to adult, was also affected by egg-load: both sexes took significantly longer to develop as the number of eggs per seed increased.

Seed Weight.

When egg-load was manipulated by the experimenter, the correlations between survival and seed weight showed the same pattern as was found for naturally-produced egg-loads (cf. Tables 4.17 & 4.19). When there were few eggs per seed, survival tended to be _negatively_ correlated with seed weight, at 4 eggs/seed the correlation became positive, and at 8 eggs/seed total survival and seed weight were significantly positively correlated. Emergence weights and development periods of either sex were not significantly correlated with seed weight.

	EGG-LOAD				CHI-SQUARE[a]	r_s
	1 (n=50)	2 (n=45)	4 (n=49)	8 (n=50)	(2 df)	(n=147)
Mean seed weight (mg)	262	260	275	247	2.37 [NS]	-0.81 [NS]
Survival (%)	78	72	66	84	15.12 [***]	-0.32 [***]
Adult sex ratio[b]	0.51	0.52	0.42	0.47	1.51 [NS]	-0.08 [NS]
Male emergence weight (mg)	4.08	3.98	3.87	3.63	5.43 [(*)]	-0.20 [*]
Female emergence weight (mg)	6.05	6.12	6.02	5.20	0.70 [NS]	0.00 [NS]
Male development period (days)	26.6	26.9	27.3	24.1	4.05 [NS]	+0.21 [(*)]
Female development period (days)	26.8	27.8	27.8	24.5	7.09 [*]	+0.24 [NS]

Table 4.18 Effect of Egg-Load on Offspring Fitness for Manipulated Egg-Loads Between 1 and 8 Eggs/Seed.

(a) Chi-square values refers to Kruskal-Wallis trends tests; both these and correlation coefficients are presented in order to increase the possibility of detecting any Allee effect (see text). (b) sex ratio = number of male offspring to emerge divided by total number of offspring to emerge. Development period refers to the period between an egg being laid and an adult subsequently emerging. NS = $P>0.1$; (*) = $P<0.1$; * = $P<0.05$; *** = $P<0.001$.

	EGG-LOAD			
	1 (n=50)	2 (n=45)	4 (n=49)	8 (n=50)
Survival	-0.11 [NS]	-0.07 [NS]	+0.02 [NS]	+0.37 [**]
Adult sex ratio$^{(a)}$	-0.35 [*]	+0.01 [NS]	-0.01 [NS]	-0.03 [NS]
Male emergence weight	+0.16 [NS]	+0.14 [NS]	+0.08 [NS]	+0.28 [NS]
Female emergence weight	+0.07 [NS]	-0.02 [NS]	-0.05 [NS]	+0.23 [NS]
Male development period	+0.06 [NS]	-0.09 [NS]	+0.13 [NS]	-0.10 [NS]
Female development period	+0.15 [NS]	+0.24 [NS]	-0.02 [NS]	-0.13 [NS]

Table 4.19 Spearman Rank Correlation Coefficients Between Seed Weight and Offspring Fitness for Manipulated Egg-Loads Between 1 and 8 Eggs/Seed.

(a) sex ratio = number of male offspring to emerge divided by total number of offspring to emerge. NS = not significant; * = P<0.05; ** = P<0.01

In summary, for both natural and manipulated egg-loads, the correlations between seed weight and survival were similar: they were negative at low egg-loads, became positive at about 4 eggs/seed and became significantly so at egg-loads above 6 eggs/seed. Because the trends were similar for both natural and manipulated egg-loads, this suggests that females probably distribute high and low quality eggs more or less randomly with respect to seed weight.

DISCUSSION

Cues for Seed Value Assessment.

Female C. maculatus distinguished between seeds differing in weight by as little as 50 mg (Expts. 4.1 and 4.6). A manipulation experiment indicated that surface area, rather than seed weight, is the main cue used by ovipositing females to discriminate between pristine seeds differing in value. For an animal assessing the size of an object that is larger than itself the use of surface area as a cue makes sense, because the use of alternative cues, such as seed weight and curvature, would probably require the development of special manipulative and/or cognitive skills; surface area has the advantage that it could be easily measured during periods when the female is presumed to be assessing the egg-load of the seed (see below). Its disadvantage as a cue is that it can only approximate seed weight. This point is illustrated by the fact that although females lay more eggs on the largest seeds they tend to underestimate the weight of large seeds and, consequently, larvae from small seeds suffer higher mortality.

A Common Decision Rule for Egg-Load and Seed Weight Discrimination: The 'Oviposition Until Inhibition' Model.

The results presented above, and those reported earlier for egg-load assessment (Introduction), suggest that there may be a common mechanism for the assessment of egg-load and seed weight. Such a mechanism is predicted if the proposition is accepted that natural selection will be parsimonious in design.

After mounting a seed, females 'inspect' it for approximately 70 s before laying an egg (Fig. 4.3). Following oviposition, locomotory activity (now known as 'march') is resumed for a similar period before a second egg is laid and the cycle repeated. Because there is no difference in the duration of the 'inspect' and 'march' phases, this suggests that the same activities are performed during each and that a single 'assessment' phase should be defined. The duration of the 'assessment' phase is possibly determined by the length of time it takes for a mature egg to move into position prior to oviposition.

It is envisaged that between each oviposition females re-assess the egg-density of the seed and stop ovipositing (and leave the seed) only when the estimated egg-load rises above a threshold value. Females are more likely to encounter eggs on small seeds than on large, and therefore their estimate of the current egg-load is likely to exceed the threshold value sooner on small seeds, resulting in the smallest clutch sizes being on the smallest seeds.

Seed weight assessment is therefore accounted for by an egg-load assessment model. It should be noted that the female, by adding to the egg-load of the seed herself, is inducing her own departure from the seed, by pushing her egg-load estimate closer to the threshold value. This "Oviposition Until Inhibition" (OUI) model is based on an absolute rule and although it can account for most of the observed trends in *C. maculatus* oviposition behaviour, it cannot explain the apparent use of a relative rule for seed weight, and possibly egg-load, discrimination (see also Table 6.1).

Decision Rules for Seed Value Discrimination.

Analysis of mean egg-laying propensities indicates that *C. maculatus* females are using an absolute rule for *egg-load* discrimination. However, the difference in clutch size on medium egg-load seeds in high and low mean egg-load environments was in the direction predicted by a crude relative rule model ($P < 0.2$), and the observed oscillations in clutch size with respect to clutch number were also in the direction predicted by a relative rule model in which the mean egg-load is learned. The conclusion that bruchids use an absolute rule for egg-load discrimination must therefore be a tentative one.

The implication of a coarse relative rule for *seed* weight discrimination is much clearer: females laid significantly more eggs on medium-sized seeds when they were paired with small seeds than when they were paired with large seeds. The oscillations in clutch size with respect to clutch number further support the use of a relative rule in which the mean seed size is learned.

In conclusion, the observed oscillations in clutch size on both egg-free and egg-laden seeds are consistent with the utilisation of some sort of relative rule, but the absolute egg-load of the seed has a major influence on clutch size decisions.

The Use of Absolute and Relative Decision Rules.

Female C. maculatus appear to be selected to maximise the rate at which they produce grand-offspring (given certain constraints; Ch.3). Implicit in a rate-maximising model of this sort is an assumption that animals respond to the mean value of their environment. However, relatively few studies to date have explicitly tested this assumption. Those that have generally support the assumption (e.g. Krebs et al. 1974, Hubbard & Cook 1978, Waage 1979, Simbolotti et al. 1987). In other words, most have shown that the study animal behaved as if using a relative rule. There are at least two exceptions to this trend however.

The first is a study by Charnov et al. (1981) on the sex ratio decisions of Heterospilis prosopoidis, a braconid wasp that parasitises bruchid larvae. The proportion of male offspring produced by a female depends on the age of the current host, but not on the proportion of hosts of that age in the environment. Thus, Heterospilis uses an absolute rule to decide the sex ratio of its offspring. The second exception is a study by Ikawa & Suzuki (1982) on the egg-laying behaviour of another gregarious parasitoid, Apanteles glomeratus. Ikawa & Suzuki found that oviposition time (and hence clutch size) depended solely on whether the host had been parasitised previously, and was completely independent of previous oviposition experience. In other words, Apanteles uses an absolute rule to make clutch size decisions.

The examples given above, suggest that absolute rules of thumb are used by some animals in some situations. However, they do not appear to be very common. One reason for this becomes apparent when one considers the simulations described in Figure 4.2. In these simulations, at both high and low egg-loads, fine-discrimination absolute rules produced very uniform distributions of eggs. However, when the average egg-load was high, many seeds were visited that were subsequently rejected. For example, in the absolute rule simulation (b), approximately 11,000 seeds were visited before all 500 eggs were laid. This compares with about 1,100 visits required in the relative rule simulations (d) and (e). Hence the oviposition rate was (10 times) slower when using an absolute rule than when using a relative rule.

One would therefore not expect to find a rate-maximising 'forager' using such a rule.

There are several possible situations in which one might expect absolute rather than relative decision rules to evolve. For example:

(1) When high egg-loads are rarely encountered in the natural environment. In this situation, selection to evolve relative rules would be no stronger than for absolute rules. This is because at low egg-loads there is little difference between the two rules in the amount of time spent visiting seeds that are subsequently rejected. However, the bruchids in the present study are probably adapted to culture conditions or to the seed store environment (Ch.1). In culture, egg-loads are typically high and in seed stores they are likely to be very variable. Selection will therefore tend to favour the evolution of relative rules.

(2) When alternative strategies, such as dispersal, have evolved as a mechanism to avoid high egg-loads. Dispersal from the natal seed patch may be induced by high egg-densities during one of two periods: (a) immediately after adult females have emerged, or (b) during the larval period, when larval crowding may induce the formation of flying morphs (Utida 1972).

(3) When the cost (in terms of time, energy or egg-equivalents) of travelling between seeds is low compared to the cost of posessing the neural apparatus required for an effective memory of previous seed encounters. In this

situation, natural selection will favour the 'cheaper' absolute rule.

(4) When the consequences of the absolute rule are modified by laying experience so that it performs like a relative rule in some situations. Simbolotti et al. (1987) demonstrated that whilst the proportion of hosts attacked by the solitary parasitoid Lariophagus distengus depended primarily on the absolute size of hosts it was presented with, clutch size was also modified according to the relative size of the host. Under the classification of the present study, Lariophagus would be deemed to be using a fine-discrimination relative rule. Simbolotti and his coworkers would argue, however, that the wasps' apparently 'relative' decisions are based entirely on 'absolute' rules. Oviposition decisions, they propose, are governed by the absolute size of the current host and the absolute number of eggs remaining in the oviducts. Simulations of these rules lend qualitative support to their hypothesis.

The proposition that absolute rules may produce relative outcomes is pursued further in Chapter 6, when a new set of mechanistic models are constructed that integrate information about the female's internal state with information about her external environment.

CHAPTER 5

PHYSIOLOGICAL CONSTRAINTS: THE RATE OF EGG MATURATION AND OVIPOSITION.

Chapter 5. Physiological Constraints: the rate of egg maturation and oviposition.

INTRODUCTION.

When testing the functional models developed in Chapter 2, it was assumed that females emerged from seeds with a full complement of mature eggs (i.e. that they were proovigenic). However, Callosobruchus beetles are synovigenic (they mature eggs whilst searching for oviposition sites; Ouedraogo & Huignard 1981), and this may have profound effects on the predictions of these models. The study of physiological processes associated with oviposition, such as egg maturation, is important if precise quantitative predictions are to be made from functional or mechanistic models (see e.g. Charnov & Skinner 1984, 1985, 1988; Skinner 1985). Information about these physiological processes can be utilised in three ways.

Firstly, they can be used to describe the strategy set of the animal and hence the range of options available to it (Ch.2, Stephens & Krebs 1986). The strategy set of an ovipositing insect can be described only after the limits imposed by her egg-storing capacity and egg maturation rate have been defined: a clutch can only be as large as the number of eggs in the oviducts, and the rate of oviposition is constrained by the rate of egg maturation. Charnov &

Skinner (1988) have recently described a clutch size model in which the egg maturation rate is included as a constraint. The main prediction of this model is that females should sometimes stay on the host whilst maturing additional eggs rather than go in search of alternative hosts. The only other functional models that include the egg maturation rate as a constraint on clutch size are the dynamic programming models of Mangel (1987a, b). Qualitative tests of these models suggest that the egg maturation rate may be an important state variable influencing clutch size decisions.

The second way that information about these physiological proceses can be utilised is in the formulation of mechanistic models of oviposition behaviour. In an elegant study of the proximate control of parasitisation by the hymenopteran _Lariophagus_ _distinguendus_, Simbolotti _et al_. (1987) produced a simple mechanistic model for oviposition decisions which required no detailed memory of previous host visits, just a knowledge of the number of mature eggs in the oviducts. In synovigenic species, the number of mature oocytes available at any given moment is a function of oviposition rate, egg maturation rate, egg-storing capacity and egg resorption rate (see Ch.6). Information about these processes is therefore vital to deciphering the mechanisms of oviposition behaviour.

The third way that information about physiological constraints can be utilised, is in studies of how the 'constraints' themselves have evolved. For example, although the rate of egg maturation may not vary throughout the life of a female (and so in some circumstances may act as a constraint), it is, nonetheless, subject to natural selection and hence will evolve (unless there is no genetic variation in this trait). Natural selection will tend to favour females that mature eggs at a similar rate to that at which hosts are usually encountered (Price 1972, see also Skutch 1967, Charnov & Skinner 1988); if they mature eggs too rapidly then they may end up having to dump or resorb some of them, whereas if they do not mature eggs fast enough then they will have too few eggs on encountering a host to lay the optimal clutch size. However, as Charnov & Skinner (1988) point out, "the concept of an 'optimal' egg maturation rate is very imprecise because, the direction and nature of selection may vary from generation to generation".

Egg Maturation Rate and Oviposition Rate.

As well as lending insights into the evolution of oviposition rates, studies of egg maturation will also shed light on the physiological control of oviposition. One reason why this is of fundamental importance is that the rate of egg maturation and the rate of oviposition are two different measures of egg production which may be influenced, independently, by different factors (see Engelmann 1970 for a review). For example, in many dipteran and lepidopteran species mating stimulates egg laying but only indirectly affects egg maturation (Engelmann 1970). In other insects, such as the locust *Schistocerca gregaria*, mating has a direct effect on egg maturation *per se* (Norris 1954). The availability of oviposition sites may also have a direct or indirect effect on oogenesis. In the hymenopteran *Diadromus pulchellus* olfactory perception of host seeds is sufficient to stimulate egg maturation (Labeyrie 1964).

In the field and in seed stores, bruchids are likely to encounter local shortages of mates and oviposition sites and possibly fluctuations in temperature and humidity. Studies have shown that these factors influence the oviposition rate and lifetime egg production of bruchids (Schoof 1941, Nwanze & Horber 1976, Giga & Smith 1983, 1987, Credland 1986). However, it is only by understanding how egg maturation is affected, and hence how a female's reservoir of mature eggs alters, that the efficiency of responses can be assessed.

Aims.

The primary aim of the present chapter is to identify physiological constraints on clutch size in *C. maculatus*, and in so doing to gain insights into characteristics of the ancestral environment.

Specifically, the following questions are addressed:

(1) When is egg maturation initiated and what proportion of the total egg output is at the mature egg stage when the

female emerges from the seed?

(2) What effect does the availability of mates and seeds have on the rate at which eggs are matured and laid?

(3) Once egg maturation has been halted, can it be restarted, and if so at what rate?

(4) Can the rate of egg maturation be varied in response to the number of oviposition sites or is it an all-or-nothing response to the presence or absence of seeds?

(5) Do *C. maculatus* females resorb eggs after they have been matured, and if so when does this start?

MATERIALS & METHODS.

Experiment 5.1. Effect of Seed and Mate Availability on Egg Maturation Rate.

The aim of this experiment was to determine whether the availability of mates and oviposition sites influences the rate of egg maturation.

Four experimental treatments were set up using newly emerged virgin females (<4h old). Group 1 females were given access to a male and 2 cowpeas; group 2 females were allowed a mate but no seeds; group 3 were given 2 seeds but no male; and group 4 were denied access to both males and suitable oviposition sites. Eggs on seeds and 'dumped' eggs (those laid on substrates unsuitable for larval development, such as the sides of the container) were counted each day. Between 5 and 15 females per group (depending on availability) were dissected on days 1 to 5 and the number of mature (chorionated) eggs in their oviducts counted.

An additional forty-five females were dissected within 30 min of emerging from seeds. The number of mature and immature oocytes in their oviducts were counted, as well as the number of ovarioles per ovary.

Experiment 5.2. Effect of Storage Time on the Development Rate and Hatching Success of Eggs.

This experiment was designed to determine firstly the timing of embryogenesis relative to oviposition, and secondly the effect of varying the duration of storage on the rate of development and hatching success of eggs.

Thirty-five newly emerged virgin females were placed in 35 ml pots with a single male and left for 24 h. At the end of this period (on day 1) all males were removed and half of the females provided with 4 cowpeas each (group A), whilst the other half were denied access to seeds (group B). On day 2, the cowpeas from group A females were removed and isolated, to be replaced by 4 new seeds. On day 3 and on all subsequent days until their deaths, females from both groups were given 4 fresh seeds to replace those that had been oviposited on during the previous 24 h. Thus, the first eggs laid by group A females (on day 1) could have been fertilised a maximum of twenty-four hours previously, whereas group B females' first eggs (laid on day 3) could have been fertilised up to forty-eight hours earlier. Seeds were inspected daily to determine hatching success and length of the L1 period (defined as the time taken for the head of the first instar larva to appear).

Experiment 5.3. Effect of Previous Egg-Laying Experience on the Rate of Egg Maturation.

This experiment was performed to determine whether egg maturation could be re-started after being induced to stop by denying females seeds for several days.

There were two main experimental treatments. Females belonging to the first (group I) were given a mate plus 4 seeds each day for the duration of the experiment, whilst the second (group II) were given neither seeds nor mates. A sub-set of group II females were given seeds plus a mate on day 3 and on subsequent days (set IIa), the remainder were given neither (set IIb). In order to make the best use of the animals available, females were not dissected on every day of the experiment: females from group I were dissected on days 3, 4 and 5; half of set IIa were dissected on day 4 and the other half on day 5; and set IIb were dissected on days 3 and 5. Thus comparisons could be made between the rates of egg maturation of similar-aged females differing only in their egg-laying experience.

Experiment 5.4. Effect of Number of Oviposition Sites on Egg Maturation Rate.

In order to assess the effect of different numbers of oviposition sites on the rate of egg maturation, two groups of beetles were established: group A females were given a mate plus 1 seed on day 0, and group B females a mate plus 10 seeds. On each of the next 2 days, they were provided with 1 and 10 fresh seeds respectively, the old seeds removed and their egg-loads determined. On day 3, females from both groups were dissected and the number of mature eggs in their oviducts counted. When added to the number of eggs that had been laid on seeds, the total number of eggs matured during the first three days could be determined.

Experiment 5.5. Effect of Female Age on Egg Resorption.

Egg-complement is influenced not only by egg maturation and oviposition, but also by egg resorption. This experiment examined the rate of egg resorption of females aged between 4 and 14 days old. One hundred and twenty newly-emerged, virgin females were retained separately in 35 ml containers without oviposition sites. On days 4, 6, 8, 10, 12 and 14 post emergence, between 14 and 32 females were dissected and the number of mature eggs in their oviducts determined. The number of eggs laid on the sides of the container was also counted.

RESULTS

Experiment 5.1. Effect of Seed and Mate Availability on Egg Maturation Rate.

Newly emerged females.

Females held 8.02 ± 3.67 (SD) mature eggs at emergence. There was no correlation between number of mature eggs and female emergence weight ($r = 0.120$, $df = 22$, NS), but the total number of immature oocytes plus mature eggs increased as female emergence weight increased ($r = 0.557$, $df = 16$, $P < 0.05$).

Number of mature eggs in the oviducts.

Both experimental treatment and female age affected the number of mature eggs held by a female (Two-way ANOVA: treatment $F_{3,164} = 66.95$, $P < 0.001$; female age $F_{4,164} = 3.64$, $P < 0.01$; Fig. 5.1), but there was no interaction between treatment and age ($F_{12,164} = 1.55$, NS). There was no difference between groups 2, 3 and 4 in the number of mature eggs carried (Tukey's comparison of means test, SAS Institute Inc. 1985). However, group 1 females (those given access to both males and oviposition sites) had fewer eggs in their oviducts than females from the other three groups

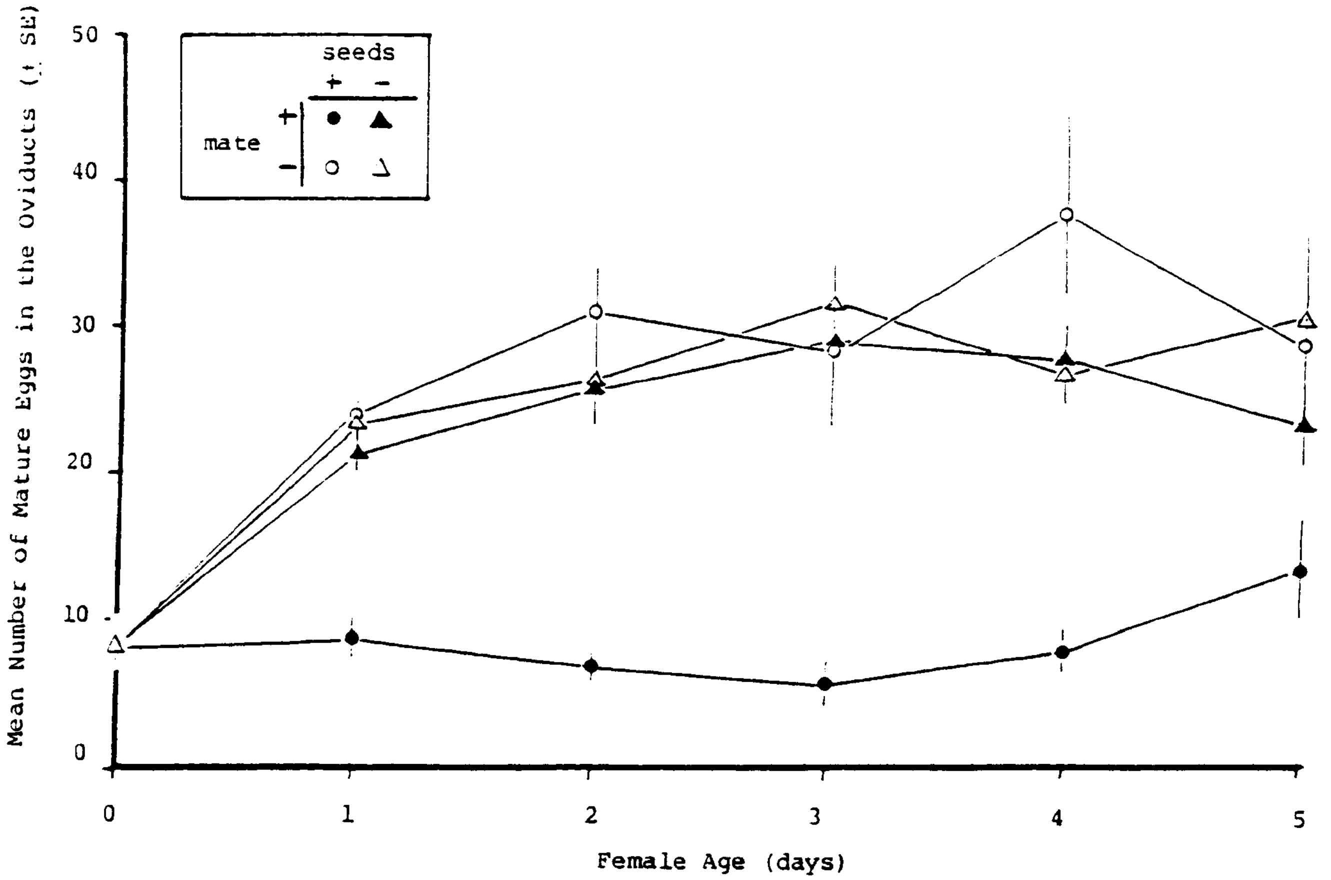


Figure 5.1 Mean Number of Mature Eggs in the Oviducts of Females Dissected on Days 1 - 5.

Each mean was calculated using 5 - 15 females.

($P < 0.05$).

Each female had between 8 and 13 ovarioles (median = 11). This variation was not correlated with weight at emergence ($r = 0.006$, df = 161, NS). The number of mature eggs held by females denied access to a mate and/or seeds remained relatively constant after day 2, and there was a significant correlation between emergence weight and the number of mature eggs in the oviducts of females belonging to groups 2, 3 and 4, dissected on days 2-5 ($r = 0.415$, df = 92, $P < 0.001$; Fig. 5.2). The correlation for day 1 alone was not significant ($r = 0.041$, df = 36, NS).

Some females 'dumped' eggs on the sides of containers (or on seeds) which could not produce viable progeny, either because the eggs were infertile (groups 3 & 4) or were laid on unsuitable substrates (groups 2 & 4). When these 'dumpers' were excluded from the analysis, the correlation between emergence weight and number of eggs in the oviducts after day 1 disappeared ($r = 0.172$, df = 42, NS). The correlation for dumpers alone was highly significant ($r = 0.662$, df = 48, $P < 0.001$). Those females that laid eggs (dumpers) were significantly lighter than those that did not ($t = 2.12$, df = 92, $P < 0.05$), and dumpers held slightly fewer eggs (27.1 ± 1.4, $n = 44$) than non-dumpers (30.0 ± 1.5, $n = 50$), but the difference was not significant ($t = 1.42$, df = 92, $P > 0.15$).

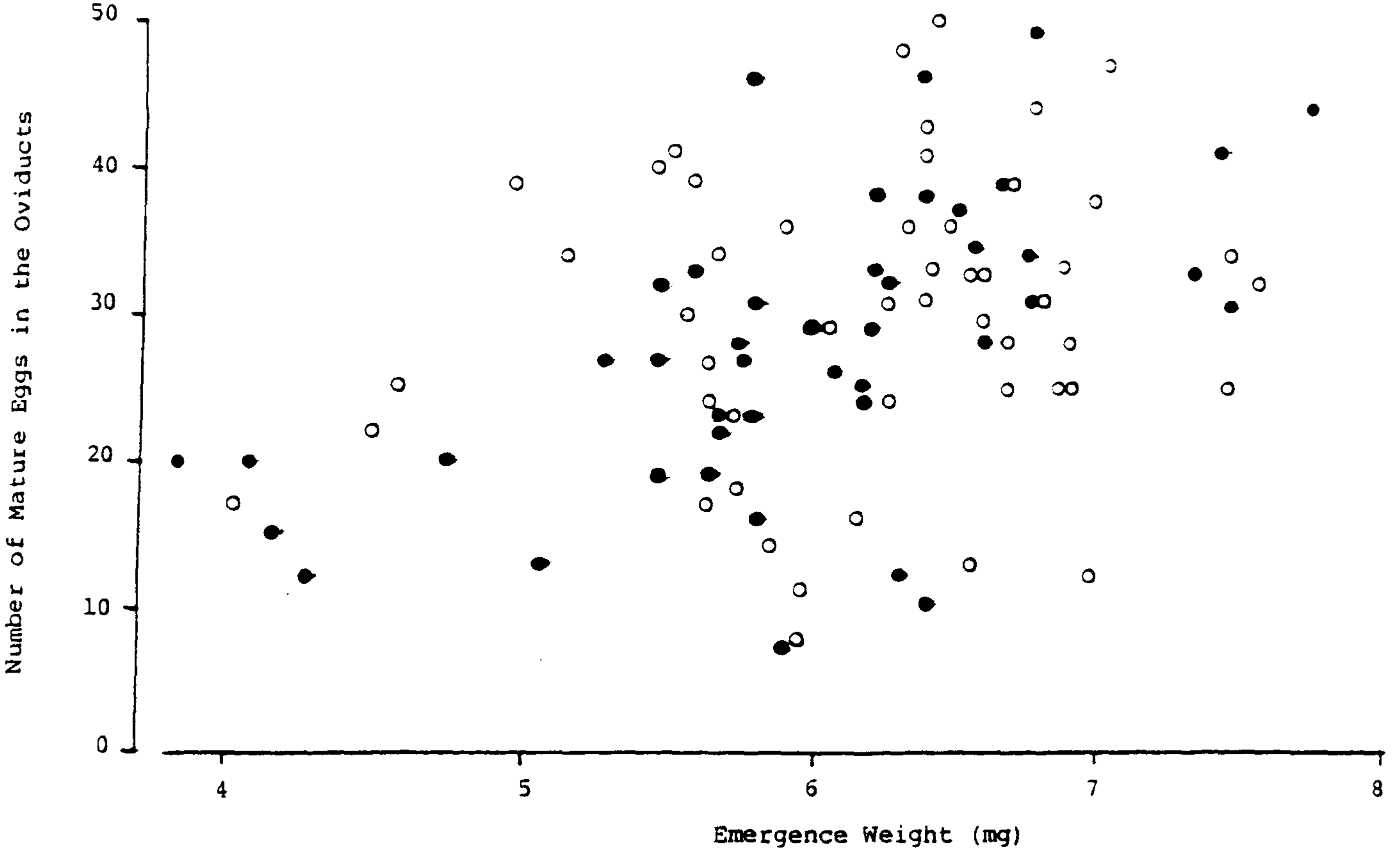


Figure 5.2 Relationship Between Number of Mature Eggs in the Oviducts at Dissection on Days 2 - 5 and Emergence Weight for Females Denied Access to a Mate and/or Seeds (groups 2 - 4).

Dumpers (○) and Non-dumpers (●) are defined in the text. Overall correlation: $r = 0.415$, $n = 94$, $P < 0.001$. Dumpers: $r = 0.662$, $n = 50$, $P < 0.001$; non-dumpers: $r = 0.172$, $n = 44$, NS.

Numbers of eggs laid.

Only females belonging to groups 1 and 3 were given seeds on which to lay. Group 1 females were also allowed to mate, and laid approximately 15 eggs per day for the first 4 days, after which time the oviposition rate decreased (Fig. 5.3; see Giga & Smith 1983 for similar constant oviposition rate over this period). Group 3 females were virgins and laid negligible numbers of eggs.

The other 2 groups were not allowed seeds, but some females laid eggs on the sides of the containers. Of the 4 groups, the only one containing females dumping a average of more than 2 eggs over the entire 5 days of the experiment was group 2. These beetles were mated but denied access to suitable oviposition sites. They dumped approximately 3 eggs per day after the day 2, and one female dumped 37 eggs over 5 days, a behaviour that demands explanation because none of these eggs will produce young.

Total egg production.

The sum of the number of eggs laid and the number remaining in the female's oviducts allows the rate of egg maturation to be calculated. There was no difference in the number of eggs matured by females belonging to groups 2, 3 and 4 (ANCOVA for days 1-5: test for homogeneity of slopes, $F_{2,126} = 1.06$, NS; test for homogeneity of intercepts,

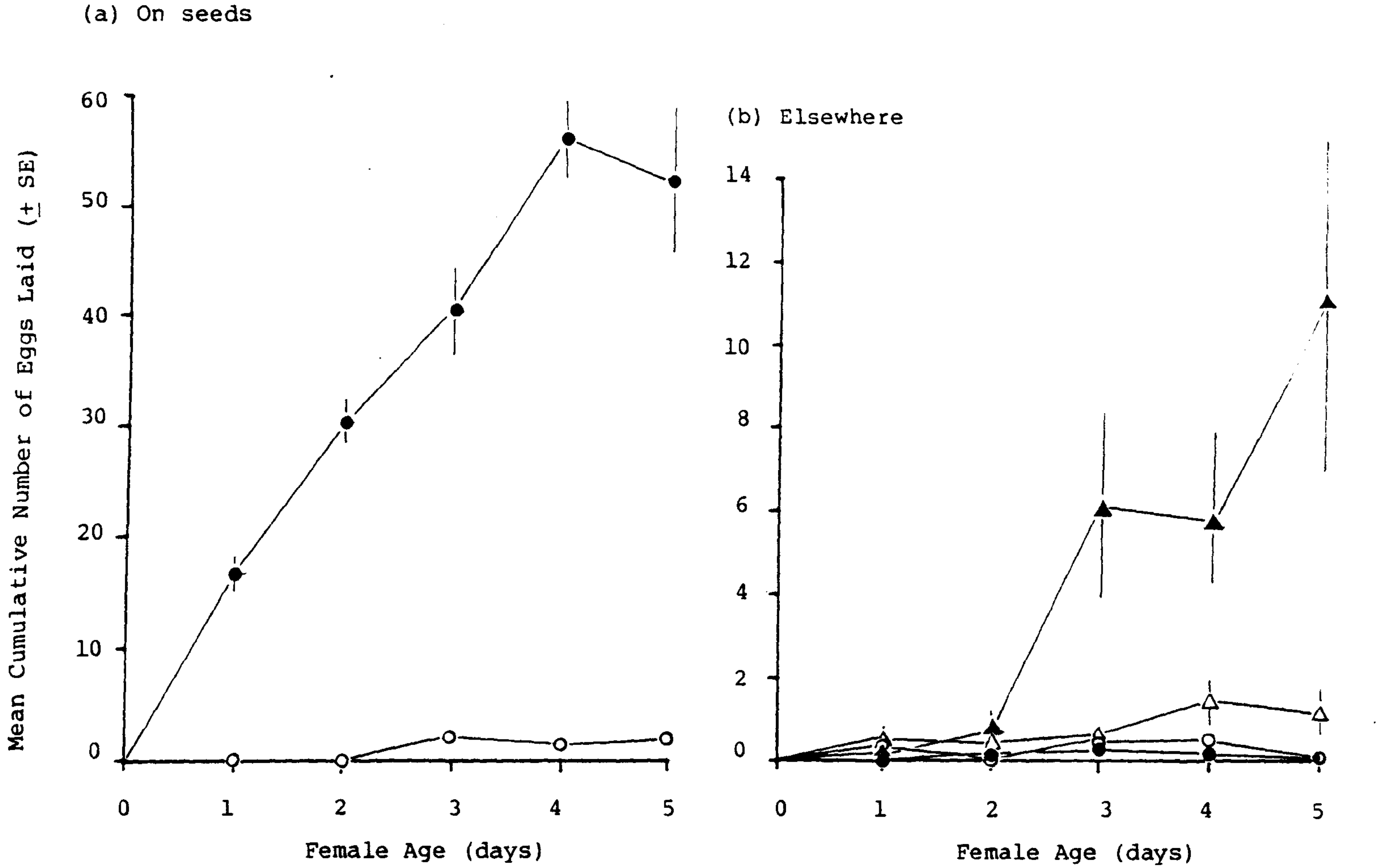


Figure 5.3 Mean Cumulative Number of Eggs Laid on Seeds and Elsewhere (e.g. Sides of Container) During the First 5 Days of Life.

Each mean was calculated using 5 - 15 females. Symbols are as for Fig. 5.1.

$F_{2,128} = 0.84$, NS; Fig. 5.4). These groups were therefore combined in an analysis comparing the regression line for females given access to both males and seeds with that for females denied either or both of these factors. The slopes of both regression lines were significantly different from zero (group 1: $F_{1,50} = 151.5$, $P < 0.001$; groups 2-4 combined: $F_{1,130} = 23.6$, $P < 0.001$), but also differed from each other (ANCOVA: test of slope, $F_{1,180} = 74.5$, $P < 0.001$; test of intercepts is therefore inappropriate). In other words, all females continued to mature eggs after day 1, but the rate of maturation was significantly higher for females belonging to group 1 than for the other 3 groups. There was no significant difference in the number of eggs matured by females from the 4 groups on day 1 (ANOVA: $F_{3,49} = 0.86$, NS), but on day 2 and on subsequent days the differences between the groups in the cumulative number of eggs matured became significant (ANOVAs: $F_{3,\geq 23} \geq 3.63$, $P < 0.05$), suggesting that it is only after day 1 that egg maturation is restrained by females belonging to groups 2-4.

Experiment 5.2. Effect of Storage Time on the Development Rate and Hatching Success of Eggs.

The first eggs laid by group A females (on day 1) started to hatch on day 5, those laid by females from group B (on day 3) began hatching on day 7. As group B females were not provided with seeds until 2 days after group A, the

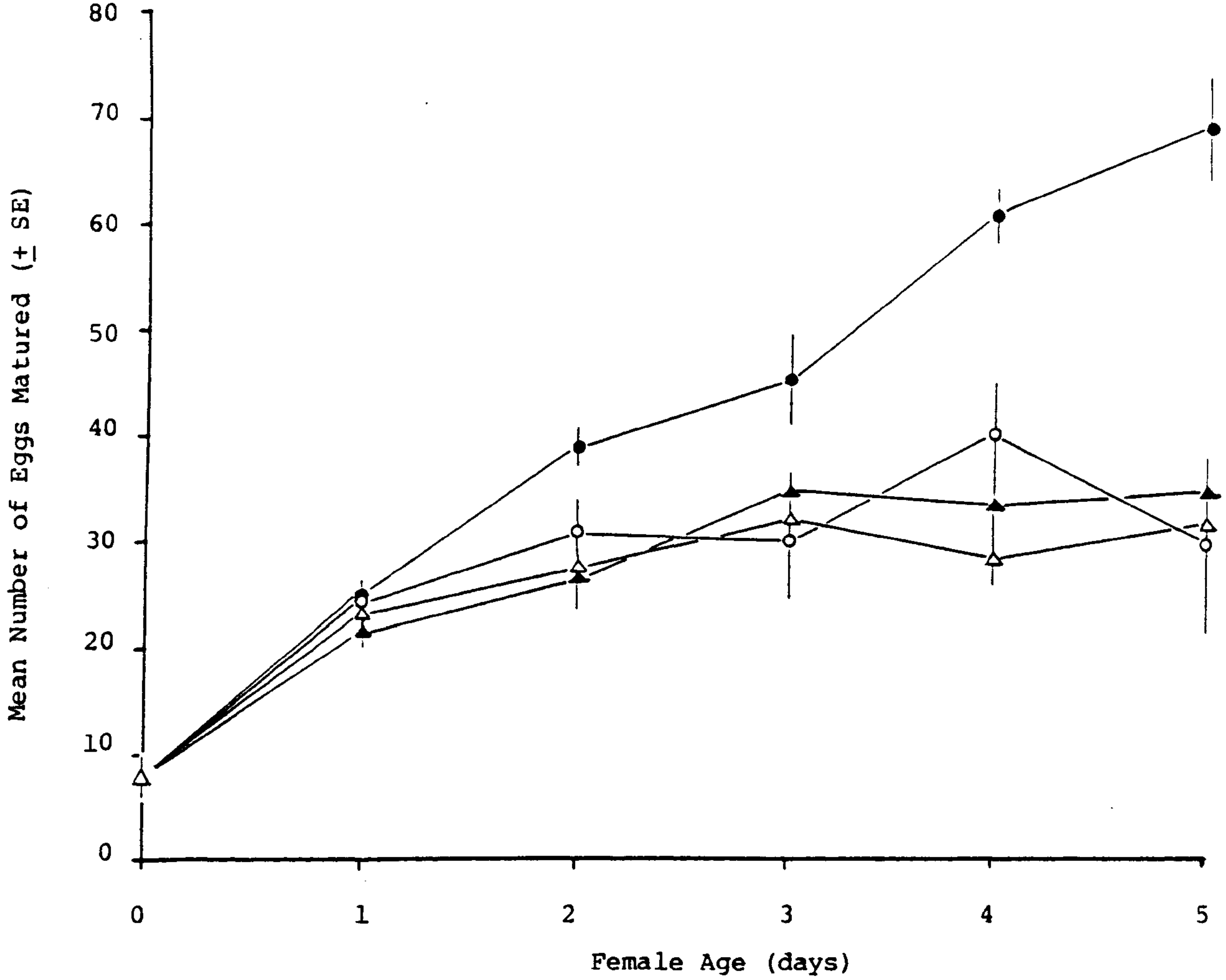


Figure 5.4 Mean Cumulative Number of Eggs Matured (Number in Oviducts Plus Number Laid) During the First Five Days of Life.

Symbols as for Fig. 5.1. Each mean calculated using 5 - 15 females.

shortest hatching period for eggs laid by both groups of females was approximately the same. Therefore, it appears that embryogenesis begins at about the same time relative to oviposition in both groups.

Temporal patterns.

A more detailed analysis showed that the mean L1 period of the 2 groups was not constant for all eggs. Those laid late in the oviposition sequence took significantly longer to hatch than those laid early on (Fig. 5.5). When the duration of the L1 period was plotted against the number of days for which females had been allowed seeds on which to oviposit, the regression lines produced for the 2 groups did not coincide. The regression coefficients were similar, but the line for group B was higher than that for group A (ANCOVA: test of slopes, $F_{1,72} = 0.49$, NS; test of intercepts, $F_{1,73} = 16.33$, $P < 0.001$). This difference disappeared, however, when the duration of the L1 period was instead plotted against female age (ANCOVA: slopes, $F_{1,72} = 0.49$; intercepts, $F_{1,73} = 0.03$, NS; note the fine dashed line in Fig. 5.5).

For both groups, mean hatching success was similar (mean percentage hatch: 82.0 ± 9.8, n = 33; t, arcsine transformed data = 0.35, df = 31, NS) and the rate of decline im hatching success did not differ (ANCOVA on arcsine transformed data: slopes, $F_{1,116} = 0.08$, NS; Fig.

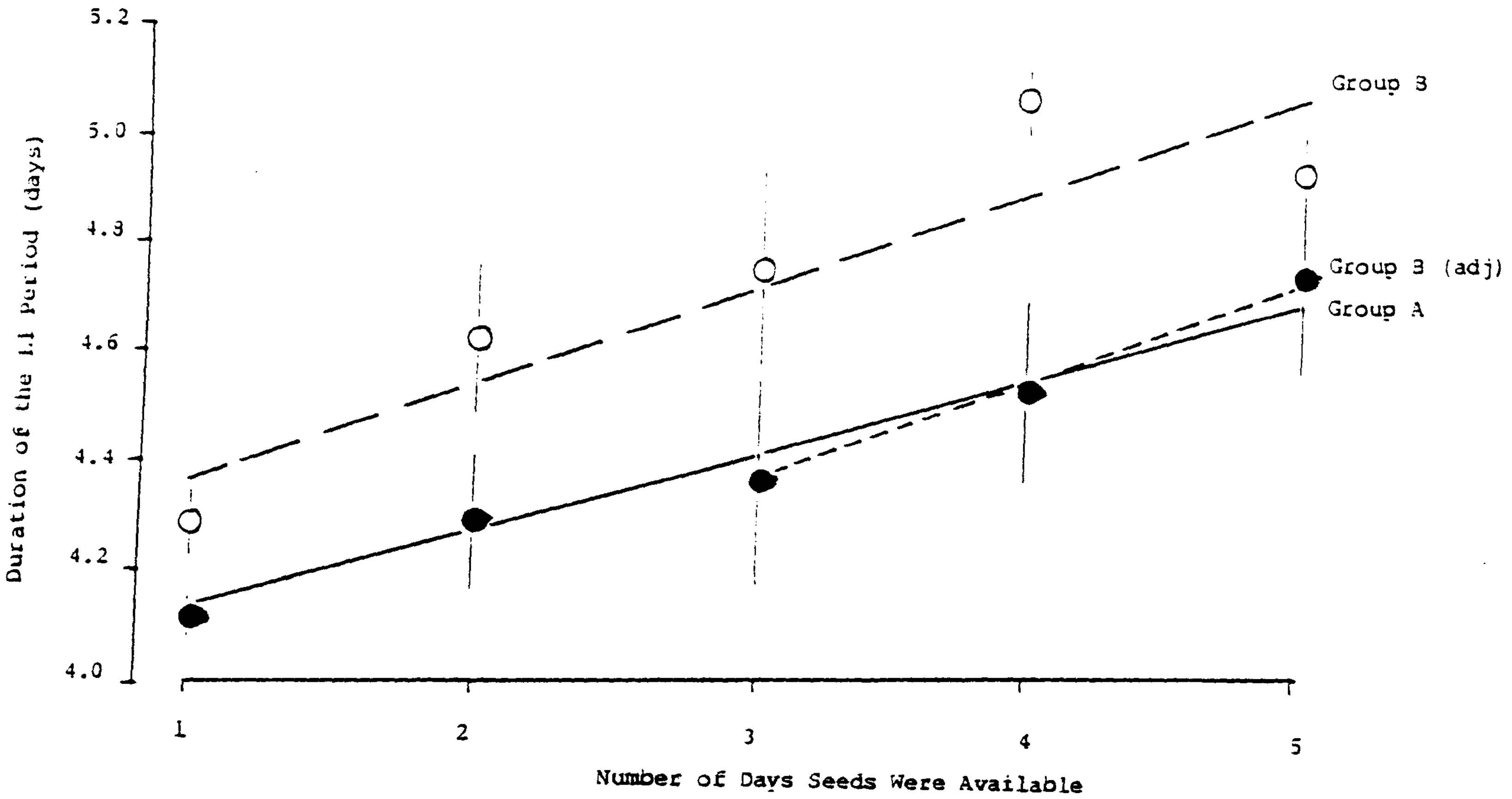


Figure 5.5 Relationship Between Mean Duration of the L1 Period and the Number of Days Since Seeds Were First Introduced.

Open symbols are Group B (given seeds from day 3); Closed symbols are Group A (given seeds from day 1). Fine dashed line is regression line for Group B females when L1 period is plotted against female age; regression line for Group A is unaltered.

5.6). The percentage of eggs which hatched declined as a function of the number of days since the female had emerged (ANCOVA: intercepts, $F_{1,117} = 0.43$, NS) rather than the number of days that seeds had been available for oviposition (ANCOVA: intercepts, $F_{1,117} = 4.79$, $P < 0.05$). In other words, both the time taken for the L1 larvae to appear and the proportion of eggs that hatched, were correlated with the age of the female at oviposition rather than at egg maturation.

Experiment 5.3. Effect of Previous Egg-Laying Experience on the Rate of Egg Maturation.

The patterns of egg maturation for females of groups I and IIb (see Fig. 5.7) were similar to those of females in the first experiment (groups 1 and 4, respectively), but the mean number of eggs matured was slightly lower. There was no significant difference between the number of eggs matured by group II females dissected on day 3, group IIa females dissected on day 4, and group IIb females dissected on day 5 (ANOVA: $F_{2,62} = 0.02$, NS). However, group IIa females dissected on day 5 had matured significantly more eggs than similar-aged females from group IIb ($t = 2.18$, $df = 69$, $P < 0.05$). Hence, it appears that egg maturation can be restarted if seeds and mates are provided, but that the response is not immediate.

The mean number of eggs matured between day 4 and day 5

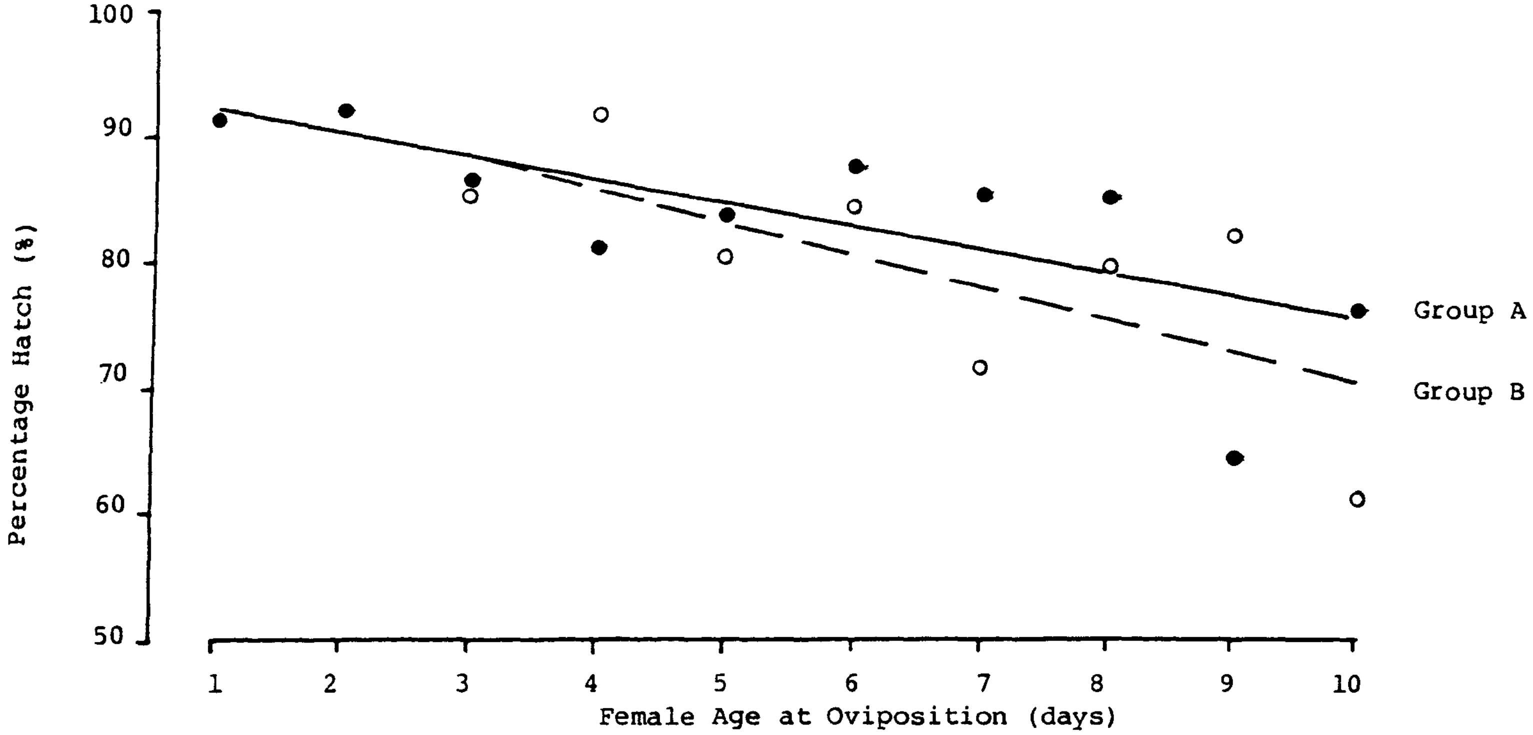


Figure 5.6 Relationship Between Percentage of Eggs to Hatch and Female Age at Oviposition.

● females given seeds on day 1; O females given seeds on day 3. Each mean was calculated from the means of 4 - 14 females. Regression analysis was performed on arcsine-transformed data (see text for details).

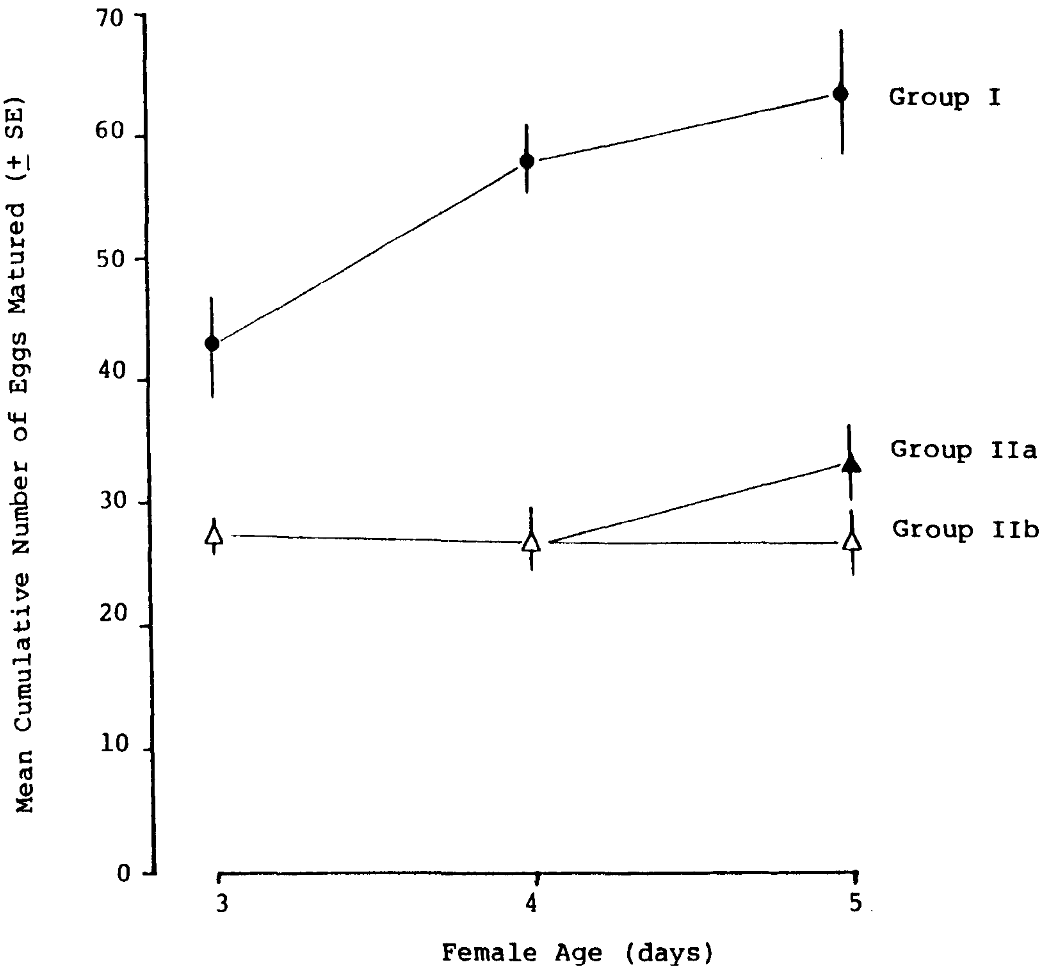


Figure 5.7 Relationship Between Mean Cumulative Number of Eggs Matured and Female Age for Females Given Seeds and a Mate on Day 1, Day 3 or Not At All.

Means were calculated using 16 - 20 different females. Females given seeds and a mate on day 1 (●), day 3 (▲), or not at all (Δ).

by females that were given seeds and a mate from day 0 (group I) was 5.9 (see Fig. 5.7). Females given a mate and seeds on day 3 (group IIa) matured 6.3 eggs over this period. Thus, when egg maturation was re-started it did so at a rate comparable with that of females that had been laying eggs since day 0. In other words, egg maturation rate, like egg fitness, was a function of female age rather than of laying experience. Although similar numbers of eggs were matured by both sets of animals between days 4 and 5, group I females laid about 5 eggs over this period, compared to about 20 by group IIa females. This indicates that, under such circumstances, the egg maturation rate is not proportional to the oviposition rate.

Experiment 5.4. Effect of Number of Oviposition Sites on Egg Maturation Rate.

During the first day of oviposition, group B females laid significantly more eggs on 10 seeds than group A females laid on a single seed ($P < 0.001$; Table 5.1). Although there was no difference between the groups in oviposition rate on days 2 and 3, the total number of eggs laid over the first three days did differ between groups ($P < 0.001$). Females belonging to both groups held approximately 6 mature eggs in their oviducts at dissection. Therefore, the total number of eggs matured by females given 10 seeds on which to lay was significantly higher than that

NUMBER OF EGGS	1 SEED (n=51)	10 SEEDS (n=55)	T	P
Laid on day 1	12.77 ± 0.78	19.36 ± 0.92	-5.45	< 0.001
Laid on day 2	15.14 ± 0.70	17.01 ± 0.84	-1.71	< 0.1
Laid on day 3	9.92 ± 0.63	9.15 ± 0.52	0.96	NS
Laid on day 1 + 2	27.90 ± 1.11	36.38 ± 1.24	-5.06	< 0.001
Laid on day 1 + 2 + 3	37.82 ± 1.53	45.53 ± 1.48	-3.64	< 0.001
In oviducts at dissection	7.63 ± 0.69	5.86 ± 1.53	2.21	< 0.05
Matured at day 3	45.45 ± 1.39	51.38 ± 1.53	-2.85	< 0.002

Table 5.1 Egg Laying and Maturation Rates (mean ± SE) of Females Given One or Ten Seeds Each Day Over Three Days.

by females given only 1 suitable oviposition site (P < 0.002). Thus, it appears that egg maturation probably is not an all-or-nothing response to suitable environmental cues, but is a graded response sensitive to oviposition rate.

Experiment 5.5. Effect of Female Age on Egg Resorption.

The number of mature eggs in the oviducts of virgin females denied seeds declined linearly between the ages of 4 and 14 days (Fig. 5.8). The number of eggs dumped was small and insufficient to account for the decline in egg-complement. This suggests that mature eggs were lost through the process of resorption. The pattern produced by superimposing data from group 1 of Experiment 5.1 indicates that resorption began between day 4 and day 5.

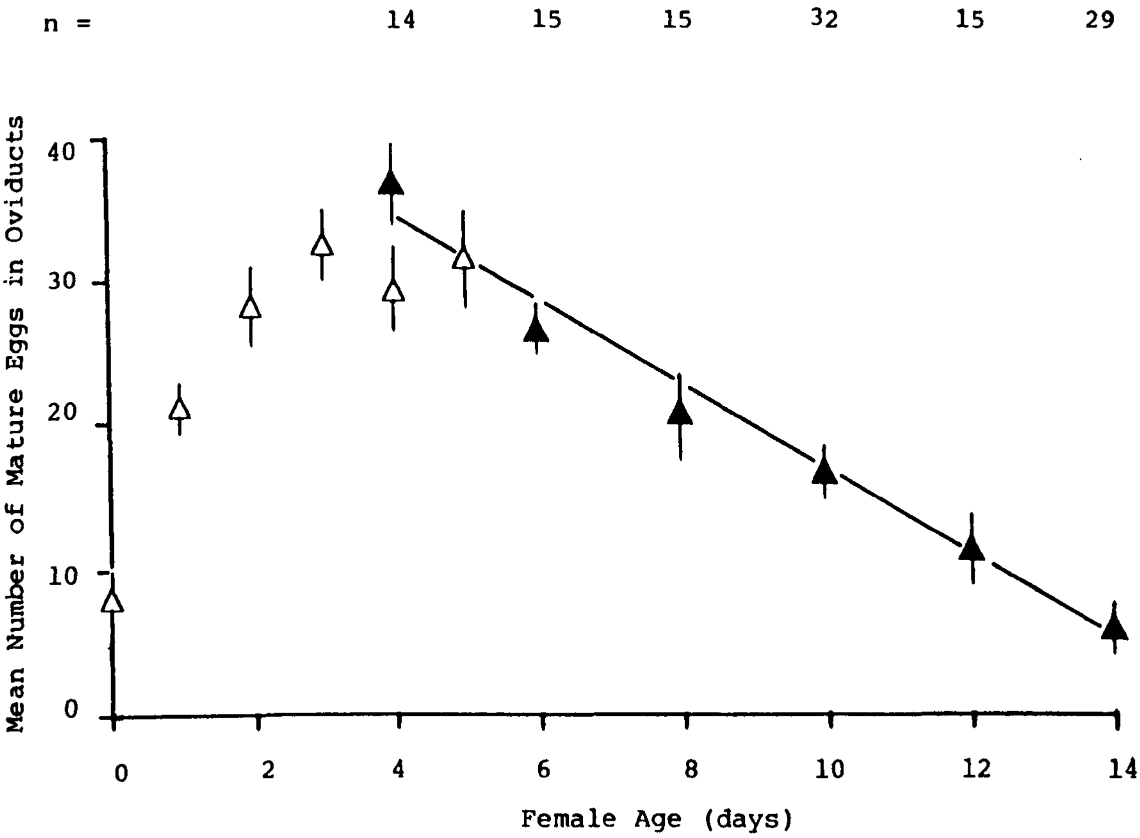


Figure 5.8 Relationship Between Number of Mature Eggs in the Oviducts and Female Age for Females Denied Access to Seeds and a Mate: The Role of Resorption.

Data are from Experiment 5.1, Group 1 (△), and Experiment 5.5 (▲).

Regression equation (for Expt. 5.5 only): $Y = 34.18 - 2.87\ X$; $F = 134.4$, df = 1,118, $P < 0.001$. Sample sizes for each mean are given above the figure. Vertical bars are standard errors.

DISCUSSION

Egg-Complement at Emergence.

Insects exhibit immense variation in the number of eggs that they have available for laying when they reach the adult stage. In some species, such as Cimex lectularius (Hemiptera), no eggs are matured unless mating first takes place (Davis 1964), whilst in others, particularly many noctuid moths, a full complement of eggs is available on the day of emergence (see Engelmann 1970). The majority of insects, however, begin adulthood with a portion of their eggs matured, and oocytes continue to develop for some time after emergence. Female C. maculatus carry about 8 eggs in their oviducts when they emerge from seeds and maintain a similar number when laying. Extrapolation of the regression line in Figure 5.4 suggests that mature eggs begin to appear in the oviducts during the day before emergence, approximately one day after the female ecloses (Bellows 1982a). Ouedraogo & Huignard (1981) obtained similar results for a population of C. maculatus that had been maintained in the laboratory for only a few generations.

The Rate of Egg Maturation.

Under the conditions of Experiment 5.1, females mature an additional 15 eggs during the day following emergence, regardless of whether seeds or mates are present. However, only mated females with suitable oviposition sites (group 1) continue to mature eggs at this rate. Females that are denied seeds or mates lay few eggs; the oviducts become packed with mature oocytes and egg maturation is inhibited. If seeds and mates become available then the rate of oviposition increases and, after a delay of approximately a day, newly matured eggs appear in the oviducts at a rate comparable with that of females that have been maturing eggs continuously since their emergence.

Credland (1986) showed that the oviposition rate of _C. maculatus_ is sensitive to the number of potential oviposition sites available. However, it is not possible to infer from his results whether the rate of egg maturation shows the same sensitivity. Results from Experiment 5.4 of the present study suggest that, under normal conditions, the rate of egg maturation is probably graded in accordance with the oviposition rate (though it may just be turned on and off at a rate that is proportional to the rate of oviposition). When mates and seeds are available, eggs are matured at the same rate as they are laid and the oviposition rate is proportional to the number of seeds. This leads to the observed relationship between the number of oviposition sites and the egg maturation rate. The availability of mates and seeds therefore has an indirect effect on the maturation rate.

Ouedraogo & Huignard (1981) found that the egg maturation rate of virgin females without seeds was lower than that for mated females or those given seeds. This suggests that both the presence of oviposition sites and mating have direct effects on oogenesis. In the present study there was no difference in the maturation rate between any of the groups (2-4) lacking mates or seeds and therefore no evidence for the effects observed by Ouedraogo & Huignard. The difference between the results of the two studies is difficult to explain.

Egg-storing Capacity.

During their lifetime, female C. maculatus may lay 80 or more eggs. However, they can only retain about half this number in their oviducts at any one time. A female's capacity for storing eggs is correlated with her body weight at emergence, but not with the number of ovarioles that comprise her ovaries, as is the case for some flies (Bennettova & Fraenkel 1981) and aphids (Wiktelius & Chiverton 1985). In the absence of seeds or mates, egg maturation is inhibited as the female approaches her capacity for storing eggs, at about day 2.

Egg Dumping.

The dumping of eggs, either by virgins or by mated females on unsuitable substrates, is common, if not ubiquitous, in insects (see Engelmann 1970). Egg-dumping is a biological phenomenon that demands explanation because it is widespread and yet appears to be maladaptive. There are at least four possible explanations for its occurence.

The first explanation is that dumping has evolved as a response to reduce the degree of egg-crowding in the oviducts below a level at which egg and/or female fitness is reduced. This hypothesis is supported by the following evidence: beetles from all three groups that were discouraged from egg-laying dumped some eggs. The correlation between emergence weight and the number of eggs in the oviducts only holds true for dumping females, suggesting that non-dumping females are those that have turned off egg maturation before reaching their egg-storing capacity. As the rate of egg maturation is independent of body weight, if maturation is turned off at the same time irrespective of female body weight then smaller individuals are more likely to reach their egg-storing capacity before halting egg maturation, and so are more likely to dump, than larger ones. In C. maculatus, dumpers were heavier than non-dumpers.

The second hypothesis is that dumping occurs because of the female's inability to retain eggs that enter the posterior portion of her reproductive tract. It is envisaged that, in preparation for oviposition, eggs move down the oviducts into an area unsuitable for their long-term storage, and that mating accelerates movement into this region. This hypothesis would account for why females who have been given a mate but no seeds dumped more eggs than any other group of females, even though all females (in groups 2-4) matured similar numbers of eggs. These first two hypotheses (i.e. that egg-dumping is an evolved response to egg-crowding, and is a constraint imposed by the egg-storing mechanism) are not mutually exclusive, and the true explanation may lie between the two.

The third hypothesis is that eggs are dumped because embryogenesis is initiated immediately after mating and the eggs would otherwise hatch within the female. Older larvae out-compete younger larvae in seeds (Bellows 1982b). Therefore, assuming that embryogenesis is triggered by fertilisation, a female that fertilised her eggs earlier relative to oviposition than conspecifics, in anticipation of finding suitable oviposition sites, would be at a selective advantage because the L1 period of her eggs would be relatively shorter. If this was correct, then females would have to dump these eggs within 4 days of mating because eggs start to hatch at 4 days old. Although eggs were dumped within this period (Fig. 5.2), the results from Experiment 5.2. do not support the hypothesis: eggs laid 2 days after maturation took as long to hatch following oviposition as eggs laid soon after maturation. This suggests that embryogenesis was initiated at the same time in all groups of females, probably immediately prior to or during oviposition (see Went 1982).

The last hypothesis considered is that eggs are dumped because after prolonged storage they have lower fitness than freshly matured eggs and that, by dumping, a female is making way for fitter eggs. Retention of ripe oocytes in the lateral oviducts has been shown to impair their later development in another bruchid, Acanthoscelides obtectus (Biemont 1979, cited by Ouedraogo & Huignard 1981). However, this did not appear to be the case in the present study: egg fitness (as measured by the duration of the L1 period and the percentage of eggs that hatched) decreased as a function of the age of the ovipositing female; the number of days that the eggs had been stored in the oviducts did not exert any additional effect. Hatching success may not be a function of female age per se but of the amount of male secretions available for utilisation in egg production (Wasserman & Asami 1985, Ouedraogo & Huignard 1981).

The entomological literature contains many reports of the apparently maladaptive behaviour of dumping. However, as yet, nobody has drawn the evidence together to suggest why there is such variability between and within species in this respect. Factors likely to influence the prevalence of dumping include: the probability of finding mates; the likelihood of locating suitable oviposition sites; the relative cost of each egg; and the potential fecundity and longevity of females. One obvious prediction is that dumping will be least prevalent in insects that do not feed as adults and for whom, therefore, each egg is likely to be relatively more costly. In this respect it is interesting to note that although virgin _C. maculatus_ dumped only a few eggs, virgin _Drosophila melanogaster_ (which feed as adults) lay just as many eggs as mated females (Partridge _et al_. 1986). The high prevalence of dumping in mated _C. maculatus_ may be because in a seed store environment mates and oviposition sites are usually located together, so that selection for mated females to restrain egg-laying is not as strong as for virgins.

Functional Models, Mechanistic Models and the Evolution of Egg Maturation Rates.

Functional models.

It was assumed in the clutch size models developed in Chapter 2 that females emerged with their full complement of eggs and that the rate of oviposition was not constrained. The present chapter clearly illustrates that these assumptions are not valid: females emerge with one-tenth of their eggs mature, they can store less than one-half of their eggs in a mature state at an one time, and it takes a minimum of 5 days to mature all of their eggs. Consequently, the rate of ovipostion is constrained and the predictions of the models may be unrealistic. Reducing the number of eggs available for oviposition will tend to decrease the optimal clutch size.

Because the number of eggs available is a function of previous oviposition behaviour, clutch size decisions are best predicted using dynamic, rather than static, modelling methods (Mangel 1987a, b, 1989).

Mechanistic models.

Simbolotti *et al.* (1987) suggested that the number of eggs in the oviducts was used by the parasitic wasp *Lariophagus distinguendus* to determine its oviposition decisions. A similar mechanism for *Callosobruchus* decision-making is implicated by the similarity between the temporal trend in clutch size when seeds are presented at daily intervals (squares in Fig. 3.7) with the temporal trend in the number of mature eggs in the oviducts of virgin females over the same period (Fig. 5.8). Mechanistic models that include internal state variables are discussed further in the following chapter (Ch.6).

Evolution of egg maturation rates.

The beetles used in the present study were of the inactive, flightless morph, which is probably adapted to life in seed stores (Utida 1972, 1981). Within stores, oviposition sites are generally abundant, but may be locally in short supply due to the presence of large numbers of conspecific females. Because eggs are continually being laid, the suitability of these sites will tend to decline over time. Consequently, natural selection is likely to favour females that are quickest to exploit conditions when they are good and that are able to respond fastest to changes in the environment.

Under the above scenario, it is predicted that C. maculatus females will be proovigenic (i.e. mature all of their eggs before emergence). In fact, only a small proportion of eggs are mature when females emerge from their seeds. It may be that to be proovigenic would extend the amount of time the female spent in the seed.

Just as turning on egg maturation prior to emergence is likely to be selected for, so is refraining from turning it off again until the oviducts are full of eggs (assuming that the cost of resorbing eggs is negligible). This is because, when competing females encounter conditions suitable for oviposition, those that have most eggs available will tend to produce most offspring. As predicted, the beetles in the present study halted egg maturation at about the same time as their egg-storing capacity was reached. The dumping of eggs by females close to their egg-storing capacity may be because these females have failed to turn off egg maturation soon enough.

The ability to quickly re-start maturing eggs will also be favoured. In C. maculatus, the response to improved conditions was not immediate; newly matured eggs appeared in the oviducts only after a delay of approximately a day. This is probably the length of time it takes for immature eggs to mature.

The predictions outlined above were for the inactive, flightless morph, but equivalent predictions can be made for the active, flight morph. This morph is adapted to colonise crops growing the field. Because it directs a large proportion of its larval reserves to flight, the active morph is less fecund than the inactive morph, and consequently, each egg is relatively more valuable. One would therefore predict that natural selection would favour females that matured their eggs only after locating a suitable oviposition site, and that dumped very few eggs. Ouedraogo & Huignard (1981) have examined some aspects of egg maturation in the flying morph of C. maculatus. They found that oogenesis began only in the presence of cowpeas, and that mating and oviposition only occured once mature eggs were present in the oviducts. No egg dumping was reported by these authors.

CHAPTER 6

MECHANISTIC MODELS FOR OVIPOSITION BEHAVIOUR: INTEGRATION OF INTERNAL AND EXTERNAL VARIABLES.

Chapter 6. Mechanistic Models for Oviposition Behaviour: Integration of Internal and External Variables.

INTRODUCTION.

In Chapter 4, models were constructed to describe possible mechanisms for discriminating between seeds differing in value (weight and egg-load). These models were based entirely on the female's assessment of the world around her; they did not include any variables related to her internal state. However, internal variables, as well as external variables, are likely to influence the motivation to oviposit. The aim of the present chapter is to identify the internal influences on clutch size decisions.

The value of any mechanistic model is determined by how closely it approximates the behaviour of real animals. The Oviposition Until Inhibition (OUI) model, developed in Chapter 4, failed to account for several important observations (Table 6.1). If the OUI model is to be rejected in favour of a model that incorporates information about the female's internal state, then the new model must provide a better description of oviposition behaviour, and account for at least some of the above discrepancies.

1. When seeds were presented to females at high frequency (at intervals up to 240 min), clutch size declined over successive clutches (Fig. 3.7).
2. When seeds were presented to females at low frequency (at intervals of 1440 min), clutch size initially increased, then levelled off, and finally decreased over successive clutches (Fig. 3.7).
3. At low host encounter rates, clutch size was primarily a function of female age, rather than of clutch number (Fig. 3.13).
4. Small females laid larger first clutches than large females (Fig. 3.9).
5. Clutch size over successive clutches sometimes exhibited oscillatory behaviour with respect to clutch number (Figs. 4.12-4.15).
6. The decision to oviposit on a seed of a given weight was influenced by the weight of the alternate seed, but the decision to oviposit on a seed with a given egg-load was apparently independent of the egg-load of the alternate seed (Tables 4.12 & 4.13).

Table 6.1 Six Discrepancies Between the Predictions of the Oviposition Until Inhibition Model and Observed Behaviour.

Previous Mechanistic Models.

Simbolotti, Putters & van den Assem (1987) recently produced a mechanistic model to describe the oviposition behaviour of the solitary parasitoid, Lariophagus distinguendus. The model they proposed integrates information about the animal's external environment (eg. size of the current host) with information regarding its internal milieu (eg. the number of mature eggs in its oviducts), to produce a behavioural tendency that results in specific behavioural options (i.e. lay a female egg, lay a male egg, or reject the host). This motivational system is potentially capable of describing the range of behaviours observed in Lariophagus, including the apparent use of a relative rule and the "erroneous decisions" described by the authors. However, Simbolotti and his co-workers did not perform any quantitative tests of their model, and their only qualitative test was a computer simulation, which indicated that relative decisions could be generated by a model of this sort. The power of the model in explaining Lariophagus oviposition behaviour must therefore remain in some doubt until more stringent tests are performed.

Physiological Variables.

Simbolotti et al. suggested that egg-complement was likely to be the most influential physiological variable (or internal state variable, in their terminology). However, there are numerous variables describing the internal state of an ovipositing female, any of which may have important influences on egg-laying behaviour. These include (a) the number of mature eggs in the oviducts, (b) the total number of eggs laid and (c) the fullness of the oviducts (number of mature eggs in the oviducts relative to the egg-storing capacity).

These variables may be estimated by the female through perception of nervous impulses generated at stretch receptors associated with the oviduct walls. Other variables, such as the number of immature eggs and the amount of reserves remaining for egg production, may also have important influences on egg-laying decisions. However, these variables are difficult to measure experimentally and so were not examined in the present study and will not be discussed further. The present chapter has two aims: firstly, to determine whether physiological variables influence the clutch size decisions of female C. maculatus; and secondly, to determine the relative importance of variables (a)-(c), above.

Assessing the Importance of Physiological Variables in Determining Oviposition Behaviour.

If the current (OUI) mechanistic model is to be superceded by one that includes physiological variables, then the new model must explain significantly more of the variation in clutch size. This can be determined in two ways. The first is to construct computer simulations of the models and to assess, qualitatively, how well they describe the observed patterns of behaviour. The second is to compare, quantitatively, the fit of GLM models that differ in the physiological variables they include. The former method examines general patterns of behaviour in a variety of situations, whilst ignoring individual variation; whereas the latter looks at the moment-by-moment decisions made by individual females that differ in various measures of their internal state.

QUALITATIVE TESTS OF THE MODELS.

The core model.

The computer simulation core model describes the temporal variation in egg-complement ($EC_{(t)}$), as a function of egg-complement at emergence ($EC_{(0)}$), egg maturation rate (**M**), egg resorption rate (**R**), clutch size laid at encounter with a host (**N**), and the egg-storing capacity (**ESC**).

Core model values $EC_{(0)}$, **M**, and **R** were obtained from Figures 5.1 & 5.8. In all simulations, egg maturation was stopped, and resorption started, when time (t) equalled 5760 min (4 days; see Fig. 5.8). However, egg maturation was halted sooner than day 4 if **EC** equalled **ESC** before then. Oogenesis was re-started only if **EC** decreased, due to oviposition, before resorption began. The **ESC** of the average female was set at 35 eggs (equivalent to that of a female weighing approximately 7 mg, at emergence; Fig. 5.2). Oviposition rate was determined by the interaction between seed value and the physiological variables (see below).

Sub-models.

Four sub-models were considered that differed in the physiological and environmental variables incorporated into the decision rules determining the clutch size to be laid

(N).

The four clutch size decision rules were:

I). N = V * 35 : clutch size depends only on the value of the current seed (V), and not on any physiological variables. This is the null model and equivalent to the OUI model.

II). N = V * EC : clutch size depends on the value of the current seed and on the current egg-complement of the female.

III). N = V * 35 * (100-L)/100 : clutch size depends on the value of the seed and on the number of eggs already laid (L); the clutch size produced is inversely proportional to the number of eggs laid.

IV). N = V * EC/ESC : clutch size depends on the value of the seed and on the proportion of the female's egg-storing capacity that is currently filled.

V may take any value between 0 and 1 (where 0 represents a small, egg-laden seed, and 1 a large egg-free seed). In the simulations, the average seed value was set at 0.18, so that when it was multiplied by the ESC for the average female (35 eggs), the resultant clutch size was 6.3 eggs/seed, approximately equal to the observed **xmax**. In all of the simulations that follow, unless otherwise stated, the first clutch was laid at t = 1080 min (18 h).

Simulations.

For each of the 4 sub-models, 6 computer simulations were performed (Simulations 1-6). These simulations mimic the experimental conditions (in Chapters 3 & 4) in which the above noted six discrepancies between predictions of the OUI model and actual behaviour were observed (Table 6.1). Female age, current egg-complement, total number of eggs laid, seed value, clutch number and clutch size were output from the simulations each time a seed was encountered (or every 24 h, whichever was more frequent). The predictions of each of the simulations were then compared with the observed behaviour of ovipositing females.

Simulation 1 determined whether the temporal variation in the egg-complement of non-laying females could be explained by just three interacting factors: the egg maturation rate, the egg resorption rate and the egg-storing capacity. The egg-complement of non-laying females was therefore determined every day until all mature eggs had been resorbed.

Simulation 2 determined whether the temporal patterns of clutch size variation at travel times between 1 and 1440 minutes (discrepancies 1 and 2, Table 6.1), could be attributed to variation in the female's internal state. Females were presented with a single seed at intervals of 10, 25, 260 or 1470 min; corresponding to travel times of 1, 10, 240 and 1440 min, respectively (oviposition times are included in the simulated intervals).

Simulation 3 analysed discrepancy 3 (Table 6.1), that at low host encounter rates clutch size was primarily a function of female age rather than of clutch number. Females were given a single seed at intervals of 1440 min (1 day), beginning at t = 1440, 2880, 4320, 5760 or 11520 min, and clutch size plotted against female age and clutch number.

Simulation 4 addressed discrepancy 4 (Table 6.1), that small females laid bigger first clutches than large females. Females capable of storing 25, 35, or 45 eggs, were given seeds at 10, 25, 260 or 1470 min intervals and their clutch sizes determined for 8 successive clutches.

Simulation 5 attempted to account for the oscillatory behaviour of clutch size with respect to clutch number (discrepancy 5, Table 6.1). At 50 min intervals, simulation-females were presented alternately with either low and medium value seeds, or low and high value seeds. In half of the simulations, the low value seed was presented first in the sequence, and in the other half, the medium or high value seed was presented first. This protocol mimics that of Experiments 4.2 and 4.5.

Simulation 6 examined discrepancy 6 (Table 6.1), that a memory of previous seed encounters was apparently used to discriminate between seeds differing in weight, but not differing in egg-load. This problem was analysed by determining which factors were important in modulating the magnitude of the difference between clutch sizes on seeds of given values in different environments. Females were presented with an alternating sequence of low and medium, low and high, or medium and high value seeds. The influence of the value of the alternate seed was assessed by plotting clutch size against clutch number for each of the seed values.

Results of Computer Simulations.

Simulation 1: Egg-complement of non-laying females as a function of female age.

Egg-complement initially increased whilst eggs were being matured at a constant rate; then remained constant after the egg-storing capacity had been reached and egg maturation had been turned off, and finally decreased after egg maturation had ceased and eggs were being resorbed. Because no eggs were laid, there were no differences in the predictions of the four sub-models. The temporal variation in egg-complements predicted by the simulations was similar to that observed for non-laying females C. maculatus (c.f. Figs. 5.8 & 6.1).

Simulation 2: Clutch size as a function of clutch number and host encounter rate.

Simulations based on sub-models II and IV (but not I or III) predicted positive correlations between clutch size and travel time (Table 6.2 and Fig. 6.2), as observed in in Experiment 3.6 (Fig. 3.8). These results provide support for the hypothesis that clutch size is determined primarily by the egg-complement of the female. At constant host encounter rates, the observed temporal pattern of clutch size

SIMULATION 1.

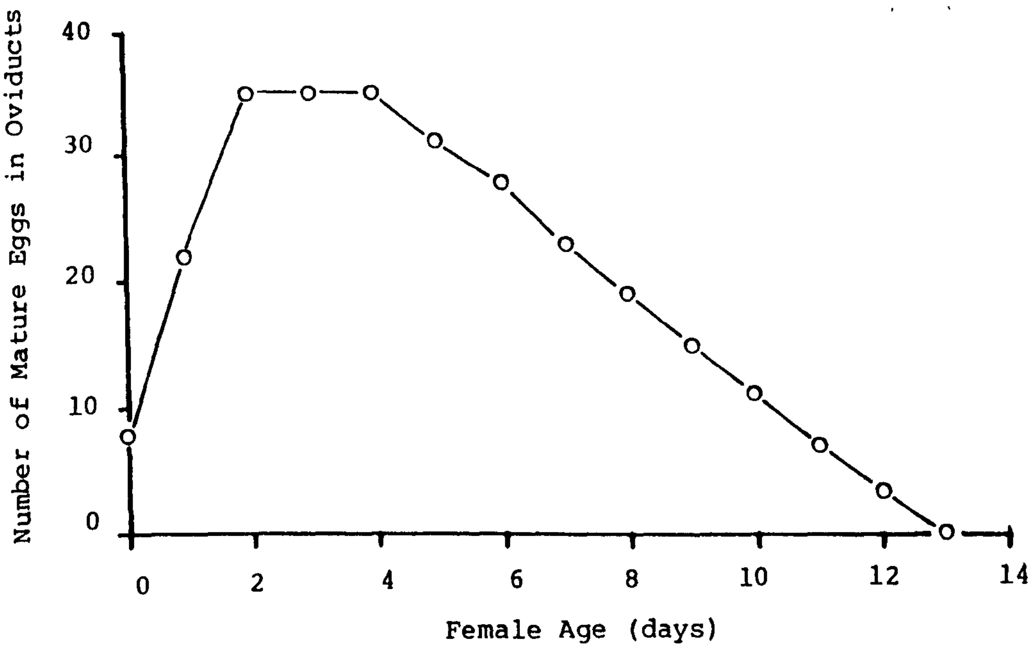


Figure 6.1 Number of Mature Eggs in the Oviducts of Non-Laying Females as a Function of Female Age Predicted by Computer Simulation of the Core Model.

Model parameters: EC(0) = 8; M = 0.0104; R = 0.0023; ESC = 35. cf Fig. 5.8, p.220.

OBSERVED TREND	SUB-MODEL			
	I	II	III	IV
<u>Simulation 2.</u>				
1. Between clutches 2-4, N was positively correlated with Tt	No	Yes	No	Yes
2. At Tt $\leq$ 10 min, N decreased over successive clutches.	N/A	Yes	N/A	Yes
3. At Tt = 240 min, N was more or less constant over first five clutches.	N/A	Yes	N/A	Yes
4. At Tt = 1440 min, N increased (between clutches 1 and 2), then was more or less constant (between clutches 2 and 4), and finally decreased (from clutch 5 onwards).	N/A	Yes	N/A	Yes
<u>Simulation 3.</u>				
5. N declined with respect to female age and clutch number.	No	Yes	Yes	Yes
6. When N was plotted against female age, treatments (day on which first seed was given) were indistinguishable.	N/A	Yes	No	Yes
7. When N was plotted against clutch number, at late clutches (numbers 4-8), N was negatively correlated with the day on which seeds were first given.	N/A	Yes	No	Yes

Cont.

OBSERVED TREND	SUB-MODEL I	SUB-MODEL II	SUB-MODEL III	SUB-MODEL IV
<u>Simulation 4.</u>				
8. Size of first clutch laid by 18 h old females was negatively correlated with female elytra length (and hence ESC).	No	No	No	Yes
9. This correlation was less pronounced or disappeared in subsequent clutches.	N/A	N/A	N/A	Yes
<u>Simulation 5.</u>				
10. N often oscillated with respect to clutch number.	No	Yes	Yes	Yes
11. N, within treatments, between seed values, tended to diverge on even-numbered clutches and converge on odd-numbered clutches.	N/A	Yes	Yes	Yes
12. N on high value seeds tended to increase on even clutch numbers; N on low value seeds tended to decrease on even-numbered clutches	N/A	Yes	Yes	Yes

Cont.

OBSERVED TREND	SUB-MODEL			
	I	II	III	IV
<u>Simulation</u> <u>6.</u>				
13. N was positively correlated with seed value.	Yes	Yes	Yes	Yes
14. N on medium value seeds tended to be larger when paired with a lower value seed (this difference was significant for seed size but not for egg-load).	No	Yes	Yes	Yes
15. Ns on high or low value seeds were not modulated by the value of the alternate seed.	N/A	No	No	No

Table 6.2 Comparisons of Observed Trends in Clutch Size with those Predicted by Simulation Sub-Models I to IV.

For details of computer simulations see text. Note that the observed trends in column 1 of this table are detailed descriptions of the discrepancies between observed and predicted clutch sizes described in Table 6.1. N = clutch size, Tt = travel time between seeds.

variation depended on the rate of host encounter: at high encounter rates (Tt $\leq$ 10 min), clutch size declined as a function of clutch number; at medium encounter rates (Tt = 240 min), clutch size remained more or less constant; and at low encounter rates (Tt = 1440 min) clutch size exhibited a domed trajectory (Fig. 3.7). Simulations based on sub-models II and IV showed similar temporal variation, adding further support to these models (Fig. 6.2). The trends predicted by the simulations are due to the temporal variation in the number of mature eggs in the oviducts: at high encounter rates, eggs are laid faster than they are matured; at medium encounter rates, oviposition rate and egg maturation rate are more or less matched; and at low encounter rate, clutch size increases initially as the egg maturation rate exceeds the oviposition rate, then remains constant as the ESC is reached, and finally decreases as eggs are resorbed faster than they are laid.

Simulation 3: Clutch size as a function of female age.

The clutch sizes predicted by sub-models II, III and IV (but not I) declined with increasing female age and clutch number (Table 6.2). However, when clutch size was plotted against female age, differences between females that started laying on different days were clearly predicted by sub-model III, but not by sub-models II or IV. Conversely, when clutch size was plotted against clutch number, differences between

SIMULATION 2.

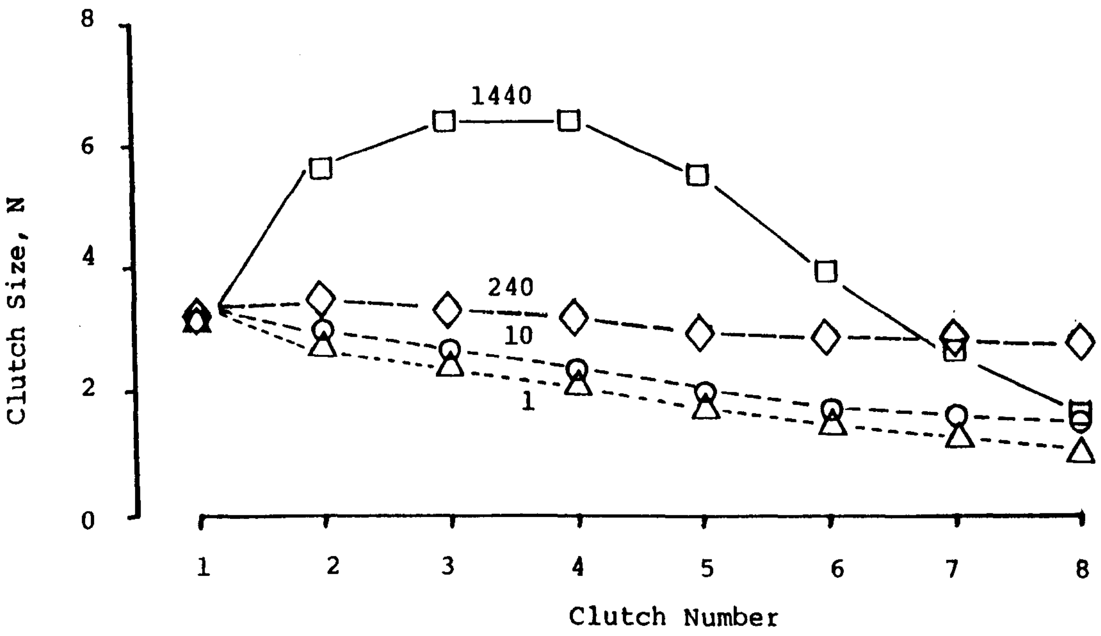


Figure 6.2 Clutch Size as a Function of Clutch Number and Travel Time Predicted by Computer Simulation of Sub-Model II.

Travel times (min): 1 (△); 10 (○); 240 (◊); 1440 (□).

Model parameters: V = 0.18; N = V*EC; EC(0), M, R, and ESC as for Fig. 6.1. Note that similar pattern is produced by sub-model IV.

cf. Fig. 3.7, p.86.

females were predicted by sub-models II and IV, but not by III (see Fig. 6.3). In other words, clutch size was predicted to be primarily a function of female age by sub-model II and IV, but of clutch number by sub-model III. The observed behaviour of ovipositing C. maculatus therefore resembles that described by sub-models II and IV rather than I or III (Fig. 3.13).

Simulation 4: Clutch size as a function of female body size.

In Expt. 3.6, the size of the first clutch was negatively correlated with female body size (Fig. 3.9). Only sub-model IV predicted this result (Table 6.2, Fig. 6.4). This is because the predicted clutch size is determined by the egg-complement of the female relative to her ESC, and so when egg-complements are the same for all females, the oviducts of small females will be fuller (i.e. EC/ESC is higher) and they will lay bigger clutches.

Sub-model I fails because it does not incorporate any variables that could account for differences between females; clutch size is determined entirely by seed value.

Sub-model II fails because all females have the same egg-complement and so lay the same size of clutch. Observed egg-complement is not correlated with female body size because the rate of egg maturation is independent of body size (see Ch.5) and, throughout the simulations, egg-complements of all females are below the ESC.

SIMULATION 3.

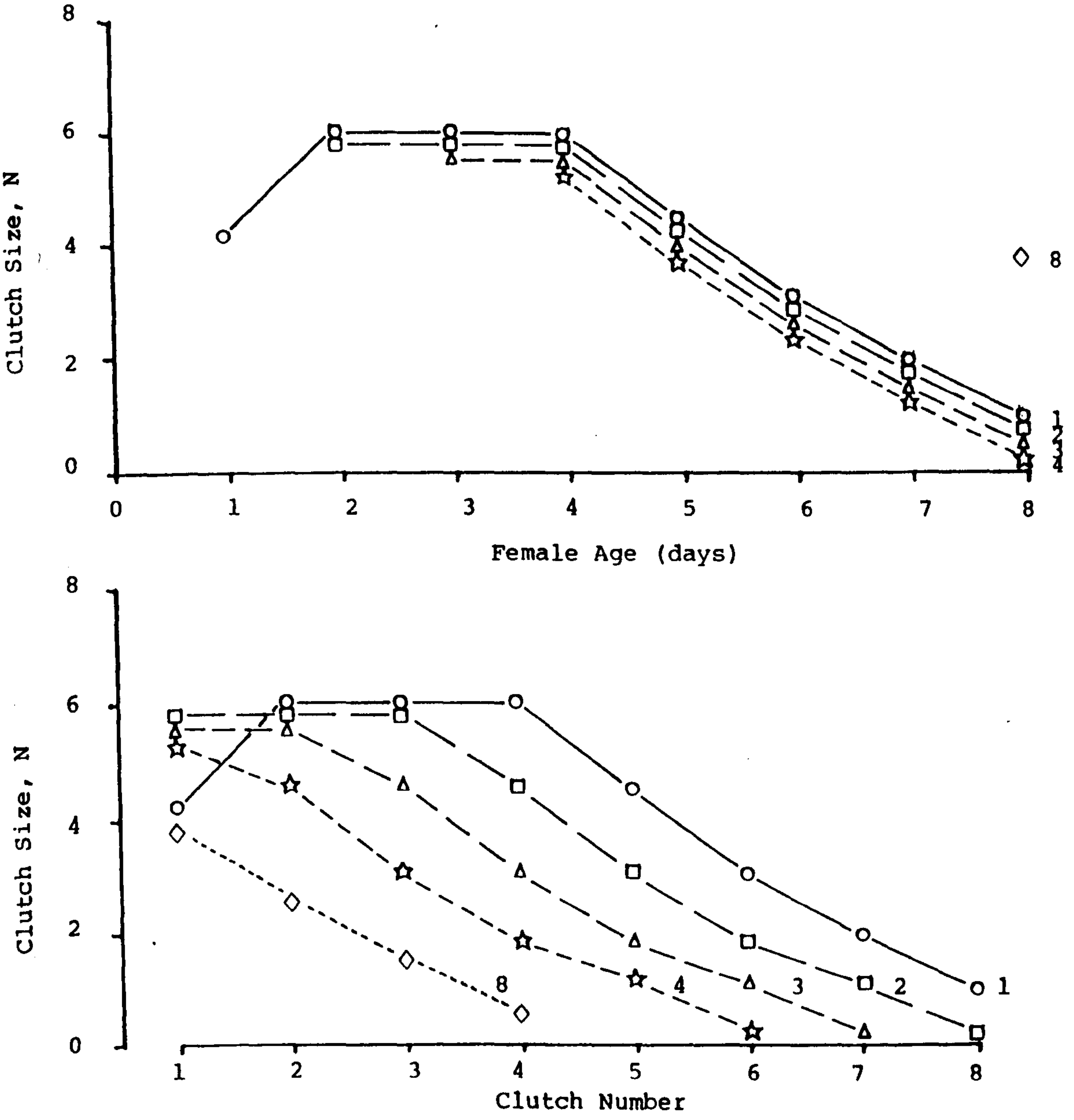


Figure 6.3 Clutch Size as a Function of Female Age and Clutch Number for Clutches Laid at Daily Intervals Starting on Day 1, 2, 3, 4 or 8 Predicted by Computer Simulation of Sub-Model II.

Females given seeds on the following days: 1 (○); 2 (□); 3 (△); 4 (☆) and 8 (◇). Note that all points shown touching have exactly the same value and are shown separated purely for clarity.

Model parameters: Tt = 1440 min; all other parameters as for Fig. 6.2. Note that similar pattern is produced by sub-model IV.

cf. Fig. 3.13, p.98.

Sub-model III fails because clutch size is determined solely by the value of the seed and the number of eggs laid, neither of which are correlated with female body size. The only predicted difference between large and small females is that small females lay fewer clutches, because they run out of eggs sooner.

It was also observed in Experiment 3.6 that although first clutches were negatively correlated with female body size, the trend did not persist to subsequent clutches (Table 3.5). Sub-model IV predicts that, at all host encounter rates, the size of the first clutch is negatively correlated with ESC and that, as eggs are laid, clutch sizes converge (Fig. 6.4). At low host encounter rates (Tt = 1440 min), the initial trend is completely reversed in later clutches and large females then lay larger clutches than small females (Fig. 6.4(c)).

Simulation 5: Clutch size as a function of previous laying experience.

When female *C. maculatus* were presented with relatively high and low value seeds in an alternating sequence (Expts 4.2 & 4.5), clutch size was sometimes observed to oscillate with respect to clutch number: the difference between clutch sizes on high and low value seeds tended to be greatest on even-numbered clutches and least on odd-numbered clutches. Even-numbered clutches tended to be larger than odd-numbered

SIMULATION 4.

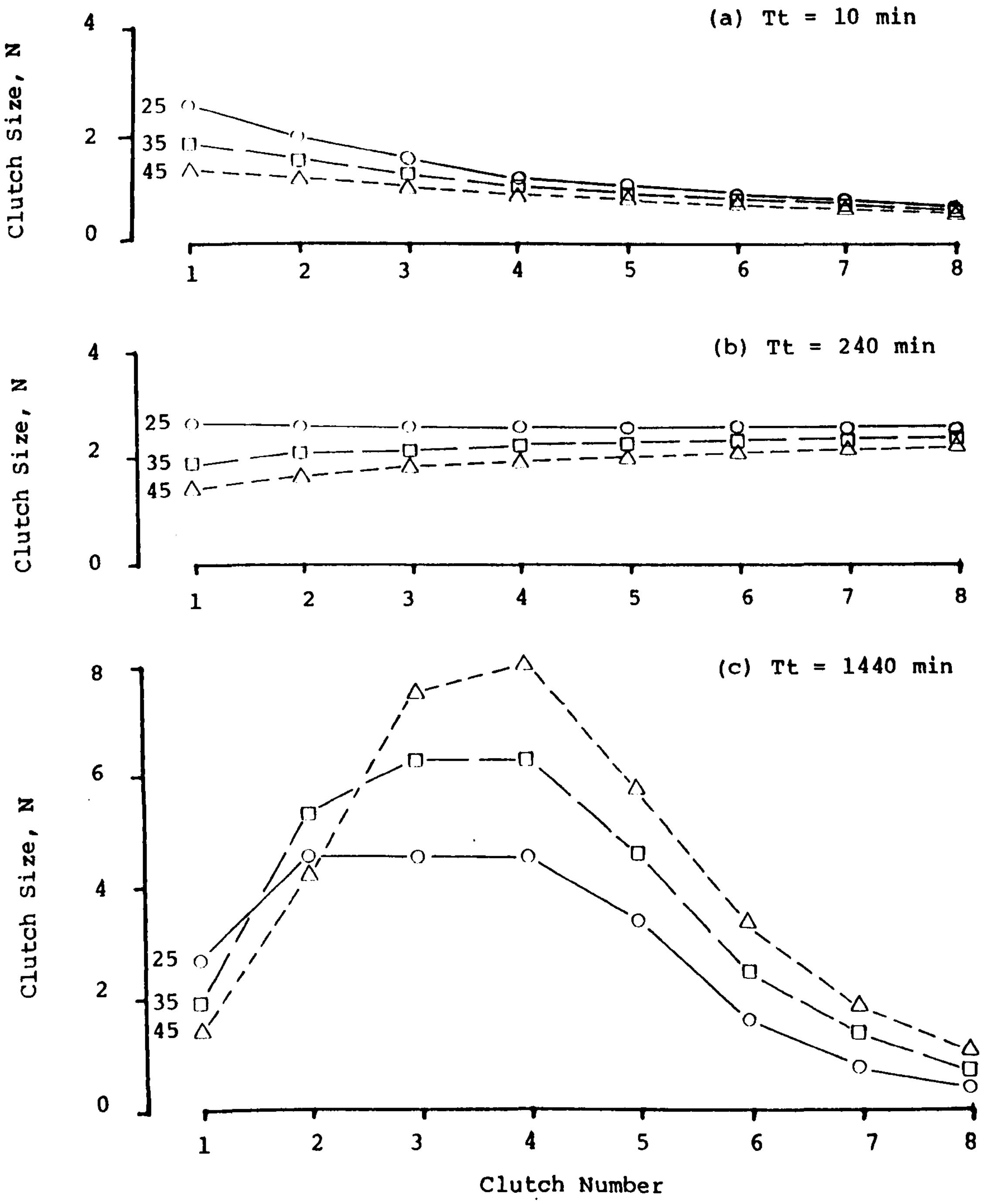


Figure 6.4 Clutch Size as a Function of Clutch Number and Egg-Storing Capacity at Travel Times of 10, 240 and 1440 minutes Predicted by Computer Simulation of Sub-Model IV.

Egg-storing capacities: 25 eggs (○); 35 eggs (□); 45 eggs (△). Model parameters : N = V * EC/ESC; parameters not already given are the same as for Fig. 6.2. Note that only sub-model IV produced the trends depicted above.

See Fig. 3.9., p.88.

clutches when the alternate seed was of relatively low value, and smaller when the alternate seed was of high value. These same patterns were predicted by sub-models II, III and IV (but not I; see Table 6.2 and Fig. 6.5).

Oscillations were predicted by sub-models II and IV because females presented with high value seeds on odd-numbered clutches ('odd' females) had, prior to laying on high value seeds, encountered equal numbers of high and low value seeds, and hence had laid equal numbers of large and small clutches. However, females given high value seeds on even-numbered clutches ('even' females) had encountered relatively more low value seeds, and hence had laid more small than large clutches prior to laying on high value seeds. The result of this was that when very small clutches were laid on low value seeds, the egg-complement of 'even' females, prior to laying on high value seeds, did not differ markedly from that of 'odd' females prior to laying on high value seeds. Consequently, 'even' clutches were of similar size to the <u>previous</u> 'odd' clutch. Egg-complements of both 'odd' and 'even' females were reduced substantially by laying large clutches on high value seeds, and therefore 'odd' clutches were substantially smaller than the <u>previous</u> 'even' clutch (but of similar size to the <u>following</u> 'even' clutch). As a result, clutch size in successive clutches proceeds in steps or oscillations.

The oscillations becomes less pronounced over successive clutches as the difference in the size of

SIMULATION 5.

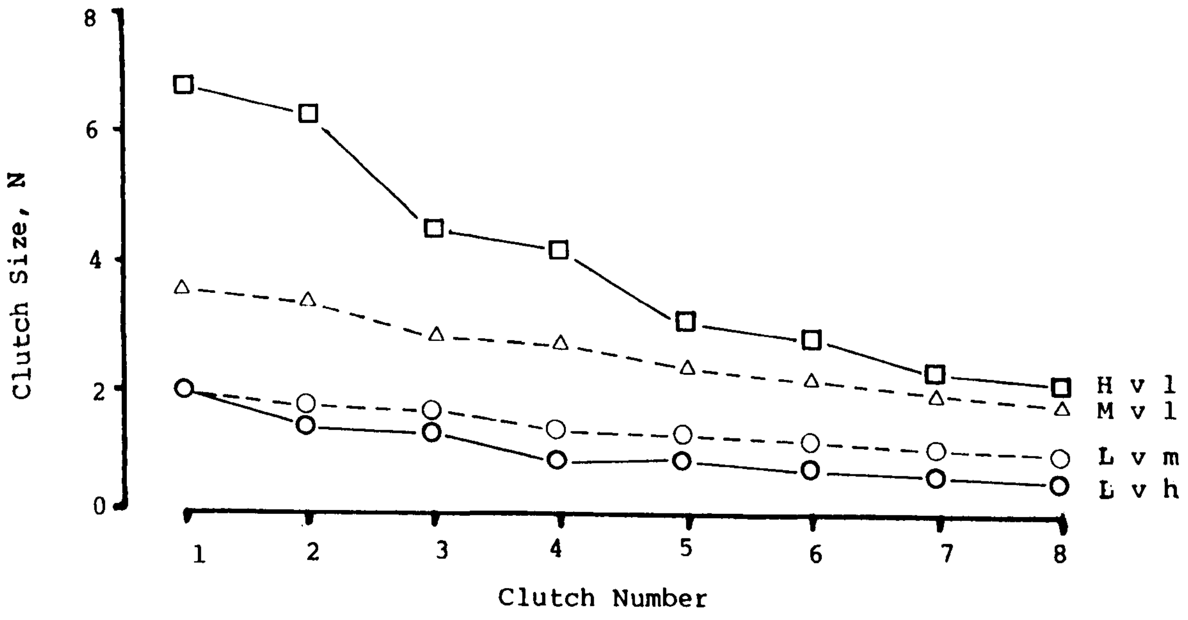


Figure 6.5 Clutch Size as a Function of Clutch Number and Seed Value when Seeds of Relatively High and Low Value are Presented in an Alternating Sequence Predicted by Computer Simulation of Sub-Model II.

Seed values: High (alternated with low, H v l) (□); M v l (△); L v m (○); L v h (O); where H, M and L are high, medium and low value seeds, respectively.

Model parameters: N = V*EC; V = 0.35, 0.18 and 0.1 for H, M and L, respectively; Tt = 50 min; all other parameters as for Fig. 6.2. Note that sub-models III and IV produce qualitatively similar results.

See Figs. 4.12-4.15 , pp.166-173.

previous large and small clutches decreases. They are reduced still further as the difference in the values of alternate seeds decreases (c.f. bold and normal symbols in Fig. 6.5(b)).

The oscillations produced by sub-model III are also due to differences between the number of eggs laid by 'odd' and 'even' females on high and low value seeds.

Simulation 6: Clutch size as a function of mean seed value.

By definition, all four sub-models predicted larger clutches on higher value seeds (Table 6.1). Under sub-models II, III and IV, clutch size was also determined by the value of the alternate seed: clutch size on higher value seeds increased as the alternate seed value decreased (Fig. 6.6). This trend was observed only for C. maculatus ovipositing on seeds of medium weight (c.f. Figs. 4.13, 4.15 & 6.6).

None of the sub-models explain the observation that females laid clutches on low and high value seeds of a size that was independent of the value of the alternate seed, or that they laid clutches of similar size on medium egg-load seeds irrespective of whether the alternate seeds were of higher or lower egg-load. However, in the simulations, the predicted response to mean seed value increased as the difference in the values of the alternate seeds, and hence sizes of alternate clutches, increased (note the small symbols in Fig. 6.6(b)). This suggests that the 'memory' of

SIMULATION 6.

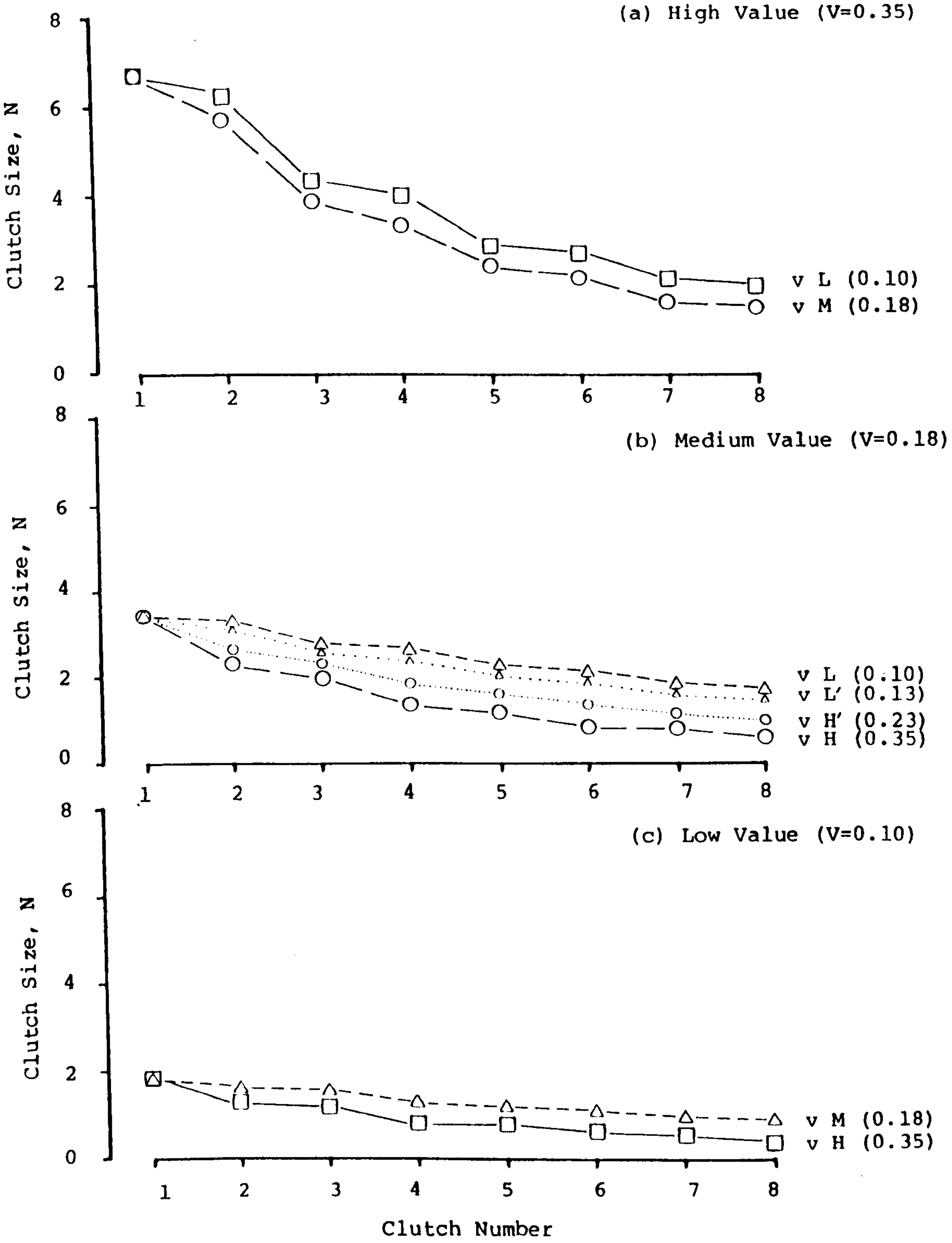


Figure 6.6 Clutch Size as a Function of Clutch Number and Value of the Alternate Seed for Seeds of High, Medium and Low Value Predicted by Computer Simulation of Sub-Model II.

Treatments: H v L (□); H v M (○); M v L (△); where H = high value, V=0.35; M = medium value, V=0.18; L = low value, V=0.10; except in (b), where the values of L′ and H′ where 0.13 and 0.23, respectively.
Model parameters: as for Fig. 6.5. Note that sub-models III and IV produce similar results.

see Figs. 4.13 & 4.15., pp. 168 & 173.

previous seed encounters is most apparent when the alternate seeds differ markedly in value, as when medium value seeds are alternated with low and high value seeds.

The apparent use of a relative rule for clutch size determination on seeds differing in weight, but not in egg-load, may be explained if the difference in clutch size on large and small seeds was greater than the difference in clutch size on 1-egg and 5-egg seeds. Unfortunately, there is little evidence for this difference (cf. Figs. 4.13 & 4.15).

Summary of Computer Simulations.

The results of the computer simulations can be summarised as follows:

(1) Sub-model I. Seed value alone can explain little of the temporal variation in clutch size.

(2) Sub-model II. Together, egg-complement and seed value can explain most of the temporal variation in clutch size, but they could not explain why small females laid bigger first clutches than large females.

(3) Sub-model III. Number of eggs laid and seed value could not explain why clutch size was modulated by host encounter rate, female age, or female body size. However, together they could explain the oscillatory behaviour of clutch size in some experiments and the 'relative' decisions.

(4) Sub-model IV. Egg-complement relative to the egg-storing capacity, combined with seed value, could explain all of the discepancies listed in the Table 6.1 except the last: Sub-model IV (like sub-models II and III) predicts that clutch size on low and high value seeds will be modulated by mean seed value.

(5) Conclusion. Physiological variables (egg-complement, number of eggs laid and relative egg-complement) predict more of the temporal variation in clutch size than seed value alone. Egg-complement relative to egg-storing capacity appears to be the best predictor of clutch size.

QUANTITATIVE TESTS OF THE MODELS.

Data from Experiments 4.2 and 4.5 were re-analysed using GLM (see Ch.4 and Appendix 2) to determine how much of the variation in clutch size could be attributed to particular physiological variables, such as the number of mature eggs in the female's oviducts, the total number of eggs she had laid, and the fullness of her oviducts.

During the 10 h that the two experiments lasted, females may have matured approximately 6 eggs (egg maturation rate = 0.0104 eggs/min; Fig. 5.8), but because it is not known whether females mature eggs whilst they are ovipositing, egg maturation during the experiment has been ignored in calculating egg-complements.

Egg-Load

The relationship between clutch size and various physiological variables was examined by comparing the GLM models shown in Table 6.3. All terms included in models 1-5 explained significant amounts of variance, and therefore lower order models (not including these terms) are not presented in the table.

Models 1, 2, 3, and 5 are equivalent to simulation sub-models I, II, III and IV, respectively: model 1 examines the role of seed value (egg-load) in determining clutch

	MODEL		SS	df_1	df_2	RMS	r^2(%)
1.	V		72.1	2	341	2.647	7
2.	V E V*E E2 V*E2	=[VE]	228.7	8	335	2.226	24
3.	V L V*L L2 V*L2	=[VL]	150.9	8	335	2.459	16
4.	[VE] L V*L L2 V*L2	=[VEL]	264.2	14	329	2.160	27
5.	[VEL] E*L E2*L E*L2 E2*L2	=[VELI]	336.8	18	325	1.963	35
6.	[VELI] F V*F F2 V*F2		357.0	24	319	1.937	37
7.	[VELI] F V*F		348.5	21	322	1.929	35
8.	[VELI] F F2		349.5	20	323	1.936	36
9.	[VELI] F		339.9	19	324	1.959	35

Table 6.3 Models Used in General Linear Modelling Analysis to Explain Variance in Clutch Size on Seeds Differing in Egg-Load (Expt. 4.5).

E = number of eggs remaining in the oviducts at dissection; V = egg-load of the seed at encounter; L = total number of eggs laid on previous seeds; F = egg-load of first seed encountered; SS = model sum of squares; df = degrees of freedom; RMS = residual (error) mean squares; r^2 = percentage of variance explained by the model.

size; model 2 examines the effect of egg-complement and seed value; model 3 examines the effect of number of eggs laid and seed value; and model 5 examines egg-complement, number of eggs laid, seed value and interactions between these factors. Egg-storing capacity cannot be easily measured, but if the egg-complement at the start of the experiment is correlated with egg-storing capacity, then model 5 considers the same sorts of factors as simulation sub-model IV.

Effect of seed value.

Egg-load alone explained 7% of the variance in clutch size ($F_{2,341} = 13.6$, $P < 0.001$; model 1, Table 6.3), but all models that included physiological variables (models 2-5) explained significantly more of the variance (tests 1-4, Table 6.4).

Effect of current egg-complement.

The addition of current egg-complement to model 1 explained an additional 17% of the variance in clutch size (24% total; model 2, Table 6.3). This result provides further support for the notion that clutch size is primarily influenced by the number of eggs in the oviducts of the female.

Clutch size tended to decrease as egg-load of the seed increased and egg-complement of the female decreased (Fig

TEST		MODELS COMPARED(1)	F	df_1	df_2	P
(a)	I	1 and 2	11.73	6	335	***
	II	1 and 3	5.34	6	335	***
	III	1 and 4	7.41	12	329	***
	IV	1 and 5	8.43	16	325	***
(b)	V	2 and 4	2.74	6	329	*
	VI	4 and 5	9.22	4	325	***
	VII	2 and 5	5.51	10	325	***
(c)	VIII	6 and 7	1.47	3	319	NS
	IX	7 and 9	2.23	2	322	NS
	X	9 and 5	1.59	1	324	NS

Table 6.4 **Statistical Comparisons of General Linear Models for Clutch Size Variation on Seeds Differing in Egg-Load** (Expt. 4.5).

(1) see Table 6.3. Interpretation of results: (a) All models that include physiological variables (egg-complement, number of eggs laid etc.) explain significantly more of the variance in clutch size than models that include egg-load alone. (b) Once egg-load and egg-complement have been included in the model, number of eggs laid and interactions between number of eggs laid and number remaining explains a substantial portion of the variance in clutch size (test VII); the significance of the interaction terms in model 5 (test VI) means that test V is invalid (see text). (c) The order in which high and low value seeds were presented to females did not explain a significant additional amount of variance. NS = $P>0.05$; * = $P<0.05$; *** = $P<0.001$.

6.7). However, two data points do not concur with these trends (asterisked in figure): when females had a large number of eggs in their oviducts (>25 eggs) or a small number (5 or less), they tended to lay smaller clutches than expected on relatively high value (1-egg) seeds. The reason for this is unclear, but suggests that seed value and egg-complement are not the only two factors involved in clutch size determination.

Effect of number of eggs laid.

The total number of eggs laid by the ovipositing female (and interactions between the number of eggs laid and the egg-load of the seed) explained 16% of the variance in clutch size (model 3, Table 6.3). This is 9% more than was explained by the null model (model 1), but 8% less than was explained by egg-complement (model 2).

Effect of initial egg-complement.

After the effects of egg-load and current egg-complement have been removed, the total number of eggs laid and interactions between the number of eggs laid and the number remaining in the oviducts explained a substantial amount (11%) of the variance in clutch size (total explained variance = 35%; model 5, Table 6.3) Because the interaction terms in the model are highly significantr (c.f. models 4

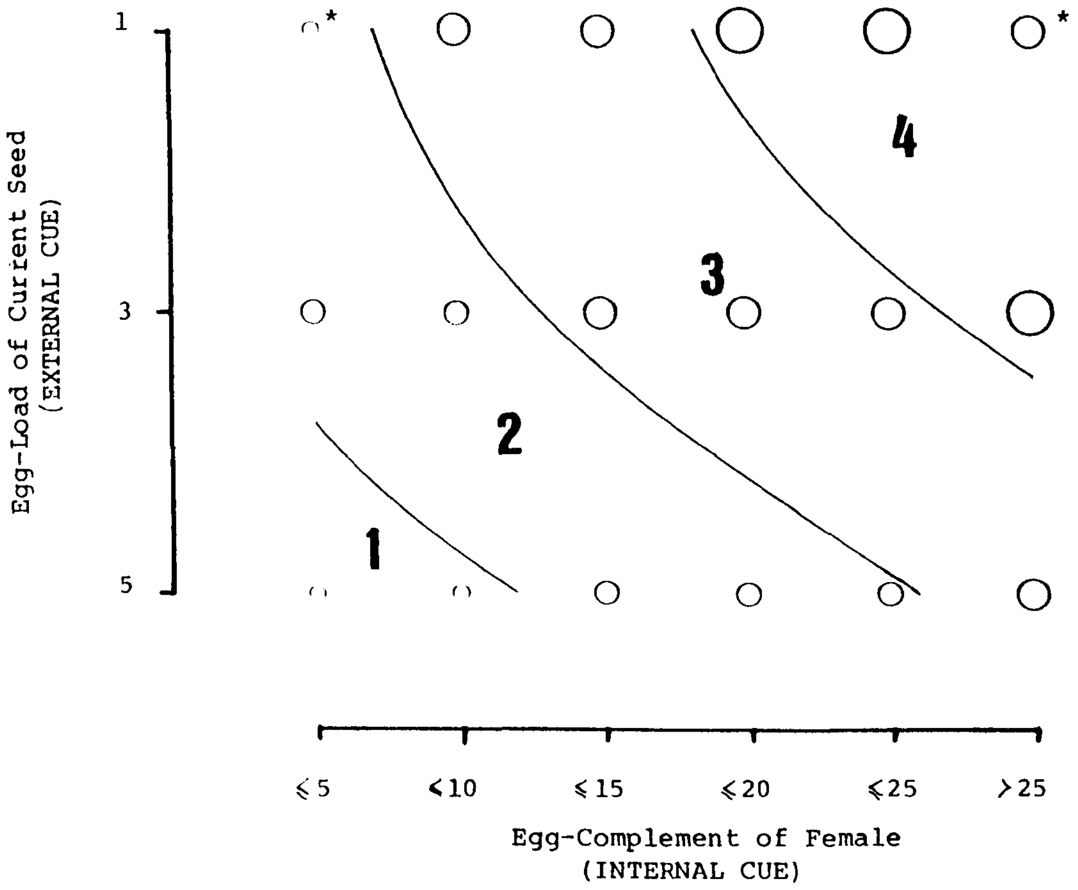


Figure 6.7 Clutch Size as a Function of Egg-Load of the Current Seed (External Cue) and Egg-Complement of the Female (Internal Cue).

Median clutch sizes are indicated by diameter of circle. The 4 sizes of circle represent clutch sizes of 1, 2, 3 and 4 eggs/seed. Diagonal lines on the figure separate areas where the combination of internal and external cue results in a given clutch size. Exceptions to this classification system are indicated by asterisks.

and 5, Table 6.3; test VI, Table 6.4), little can be interpreted from comparing models 2 and 4 directly.

The combination of current egg-complement and number of eggs laid (and interactions between the two) describes the egg-complement of the female at the start of the experiment (assuming that no eggs have been matured or resorbed during the experiment). The results presented above suggest that clutch size is determined by the egg-complement of the female relative to her initial egg-complement or ESC, but that the relationship is not a simple additional one (this is indicated by the significance of the interaction terms in the model). In Experiment 4.2, initial egg-complement was positively correlated with elytra length ($r_s = 0.336$, $n = 43$, $P < 0.05$), suggesting that initial egg-complement was correlated with egg-storing capacity and that it is egg-complement relative to the ESC that was the important determinant of clutch size in this experiment.

In Figure 6.8, clutch size is shown as a function of the estimated egg-complement of the female at each seed encounter and total number of eggs laid prior to that encounter. Because initial egg-complement is equal to the total number of eggs laid plus the number remaining at dissection, the diagonal lines in the figure join females of similar initial egg-complements. Clutch size declines as the number of eggs remaining in the oviducts decreases. Moreover, for any given egg-complement (shown on the

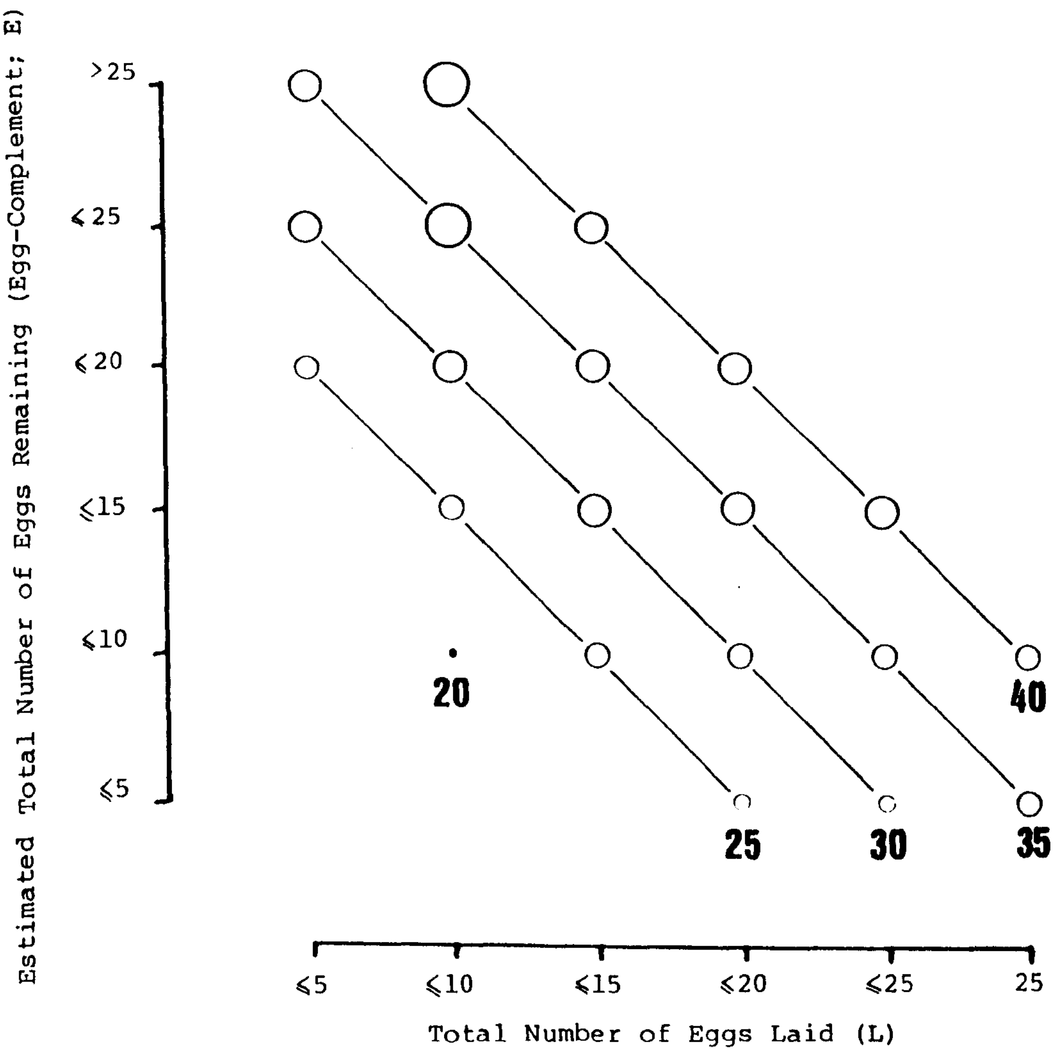


Figure 6.8 Clutch Size as a Function of Number of Eggs Remaining in the Oviducts (Egg-Complement) and Total Number of Eggs Laid.

Median clutch sizes are indicated by diameter of circle (and symbols are consistent with Fig. 6.7). The dot to the left of the figure indicates that no eggs were laid. The 4 sizes of circle represent clutch sizes of 1, 2, 3 and 4 eggs/seed. Diagonal lines join together combinations of E and L that add up to a given value (estimated egg-storing capacity). These values are shown as numbers in bold face.

vertical axis), females with the largest initial egg-complements tended to lay the largest clutches (i.e. clutch size increases from left to right).

Effect of previous laying experience.

In Experiments 4.2 and 4.5, clutch size was observed to oscillate with respect to clutch number. It was suggested earlier that these oscillations were due, primarily, to the different laying experiences of 'odd' and 'even' females (defined above). If this explanation is correct then a separate variable, representing the order in which seeds were presented to particular females, should not explain any more of the variance in clutch size than those parameters already included in model 5 (seed value, egg-complement, number of eggs laid, and interactions between all of these). Tests VIII-X in Table 6.4, suggest that no such variable need be invoked.

Seed Weight

Data from Experiment 4.5 were re-analysed using the same GLM models as for Experiment 4.2 (Tables 6.5 & 6.6).

Egg-complements were not determined at the end of Experiment 4.5, and therefore initial egg-complement was approximated by the number of eggs laid during the experiment. In order to examine the accuracy of this

	MODEL		SS	df_1	df_2	RMS	r^2(%)
1.	V		32.0	2	333	3.157	3
2.	V E V*E E2 V*E2	=[VE]	132.8	8	327	2.907	12
3.	V L V*L L2 V*L2	=[VL]	120.9	8	327	2.943	11
4.	[VE] L V*L L2 V*L2	=[VEL]	143.2	14	321	2.929	13
5.	[VEL] E*L E2*L E*L2 E2*L2	=[VELI]	235.7	18	317	2.674	22
6.	[VELI] F V*F F2 V*F2		265.2	24	311	2.631	25
7.	[VELI] F V*F		246.9	21	314	2.664	23
8.	[VELI] F F2		251.4	20	315	2.641	23
9.	[VELI] F		237.8	19	316	2.676	22

Table 6.5 Models Used in General Linear Modelling Analysis to Explain Variance in Clutch Size on Seeds Differing in Weight (Expt. 4.6).

E = number of eggs remaining in the oviducts at dissection; V = seed weight; L = total number of eggs laid on previous seeds; F = weight of first seed encountered; SS = model sum of squares; df = degrees of freedom; RMS = residual (error) mean squares; r^2 = percentage of variance explained by the model.

TEST		MODELS COMPARED(1)	F	df_1	df_2	P
(a)	I	1 and 2	5.78	6	327	***
	II	1 and 3	5.03	6	327	***
	III	1 and 4	3.16	12	321	***
	IV	1 and 5	4.76	16	317	***
(b)	V	2 and 4	0.59	6	321	NS
	VI	4 and 5	8.65	4	317	***
	VII	2 and 5	3.85	10	317	***
(c)	VIII	6 and 7	2.32	3	311	NS
	IX	7 and 9	1.71	2	314	NS
	X	9 and 5	0.79	1	316	NS

Table 6.6 Statistical Comparisons of General Linear Models for Clutch Size Variation on Seeds Differing in Weight (Expt. 4.6).

(1) see Table 6.5. Interpretation of results: as for Table 6.4 except substitute seed weight for egg-load.

approximation, all of the analyses were repeated using only those 14 females that had, apparently, laid close to their entire complement of eggs (i.e. those that laid one or no egg on on the eighth seed presented to them). These females exhibited all of the trends described below for the entire sample, suggesting that the total number of eggs laid is a good approximation to initial egg-complement.

Effect of seed value.

Seed weight alone explained just 3% of the variance in clutch size ($F_{2,333} = 5.07$, $P < 0.01$, model 1 Table 6.5), and all models that included physiological variables (models 2-5, Table 6.5) explained significantly more of the variance (tests I-IV, Table 6.6). This result is in agreement with that found earlier for clutch size on egg-laden seeds.

Effect of current egg-complement.

Current egg-complement, seed weight and interactions between these factors, explained 12% of the variance in clutch size (9% more than was explained by seed weight alone). The interaction between egg-complement and seed weight is illustrated in Fig. 6.9. The observed patterns are similar those exhibited when seed value was determined by egg-load, but clutch sizes are generally larger (as would be expected given that all seeds were initially egg-free).

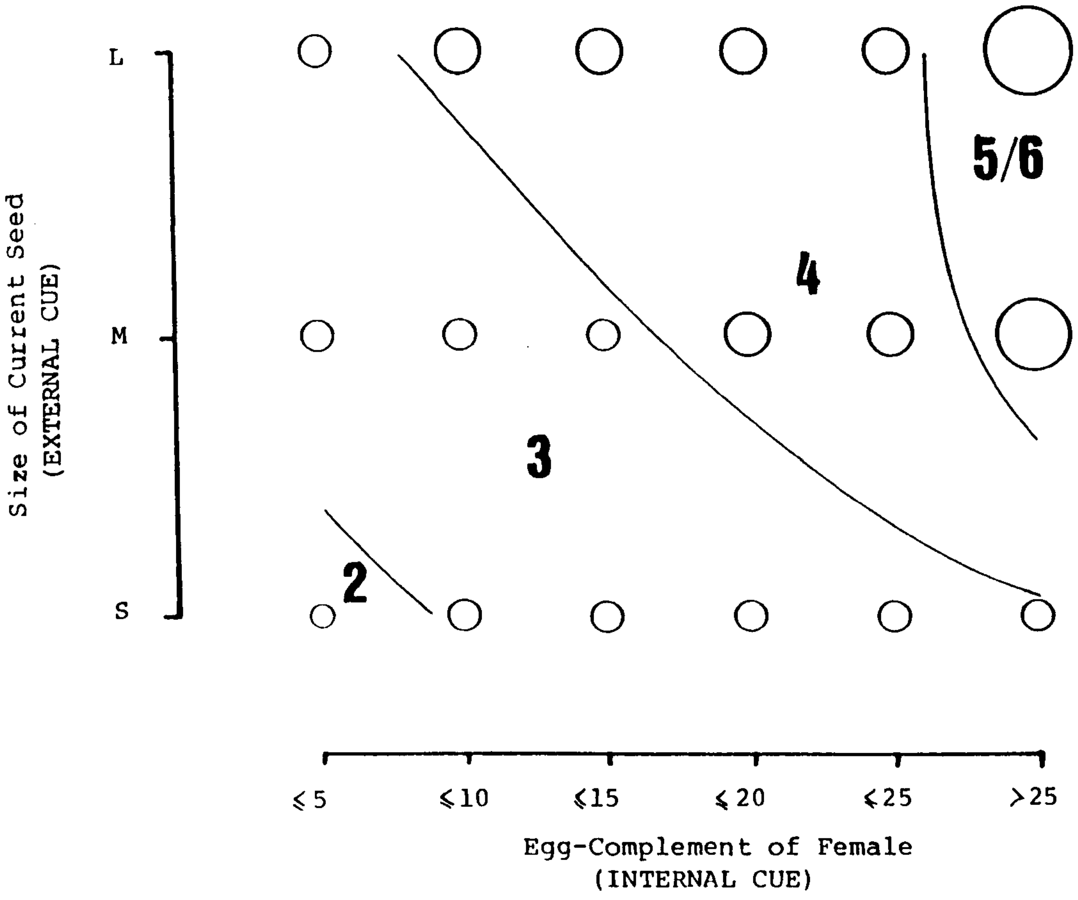


Figure 6.9 Clutch Size as a Function of the Size of the Current Seed (External Cue) and Egg-Complement of the Female (Internal Cue).

Median clutch sizes are indicated by diameter of circle. The 5 sizes of circle represent clutch sizes of 2, 3, 4, 5 and 6 eggs/ seed. Diagonal lines on the figure separate areas where the combination of internal and external cues results in a given clutch size (shown as numbers in bold face). There are no exceptions to this classification system. Note that circle diameters are consistent with Figs. 6.7 and 6.8. Seed sizes are large (L), medium (M) and small (S).

Effect of number of eggs laid.

Together, total number of eggs laid and seed weight (plus interactions between the two) explained 11% of the variance in clutch size (model 3, Table 6.5). Not surprisingly, this is approximately the same as was explained by the egg-complement model (model 2, Table 6.5). This is because, in the present analyses, initial egg-complement was estimated by the final number of eggs laid, and therefore the two are correlated. For this reason, after egg-complement and seed weight had been included in the model, number of eggs laid did not increase the amount of variance in clutch size that could be explained (test V, Table 6.6).

Effect of initial egg-complement.

Egg-complement, number of eggs laid and interactions between these factors explained an additional 10% of the variance in clutch size (c.f. models 4 & 5, Table 6.5). This result adds support to the hypothesis that clutch size is primarily determined by the relative egg-complement of the female.

In Experiment 4.2, estimated initial egg-complement was significantly correlated with female elytra length, suggesting that some females had reached their ESC by the start of the experiment. The correlation in Experiment 4.5 was not significant (all females: $r_s = 0.157$, $n = 42$, NS; 'exhausted' females: $r_s = 0.042$, $n = 14$, NS). This may indicate that most females had not reached their ESC by the start of the experiment. This is further suppoted by the fact that egg-complements of both 'exhausted' and 'non-exhausted' females in Experiment 4.5 were significantly smaller than those of females in the Experiment 4.2 ($t = 2.294$, $n = 73$, $P < 0.01$). If the mean initial egg-complement was below the ESC of most females, then initial egg-complements are probably not correlated with ESCs, and therefore no predictions can be made regarding the relative size of clutches laid by large and small females (see Simulation 4, above).

Figure 6.10 illustrates the role of initial egg-complement in determining clutch size. The pattern is not as clear as in Figure 6.8, but for 4 of the 6 egg-complement categories shown on the vertical axis a clear trend is shown for females starting with the smallest egg-complements to lay the largest clutches (i.e. clutch size declines from left to right).

Effect of previous laying experience.

After seed weight, number of eggs laid and initial egg-complement had been taken into account, the order in which alternate seeds were presented to the female did not

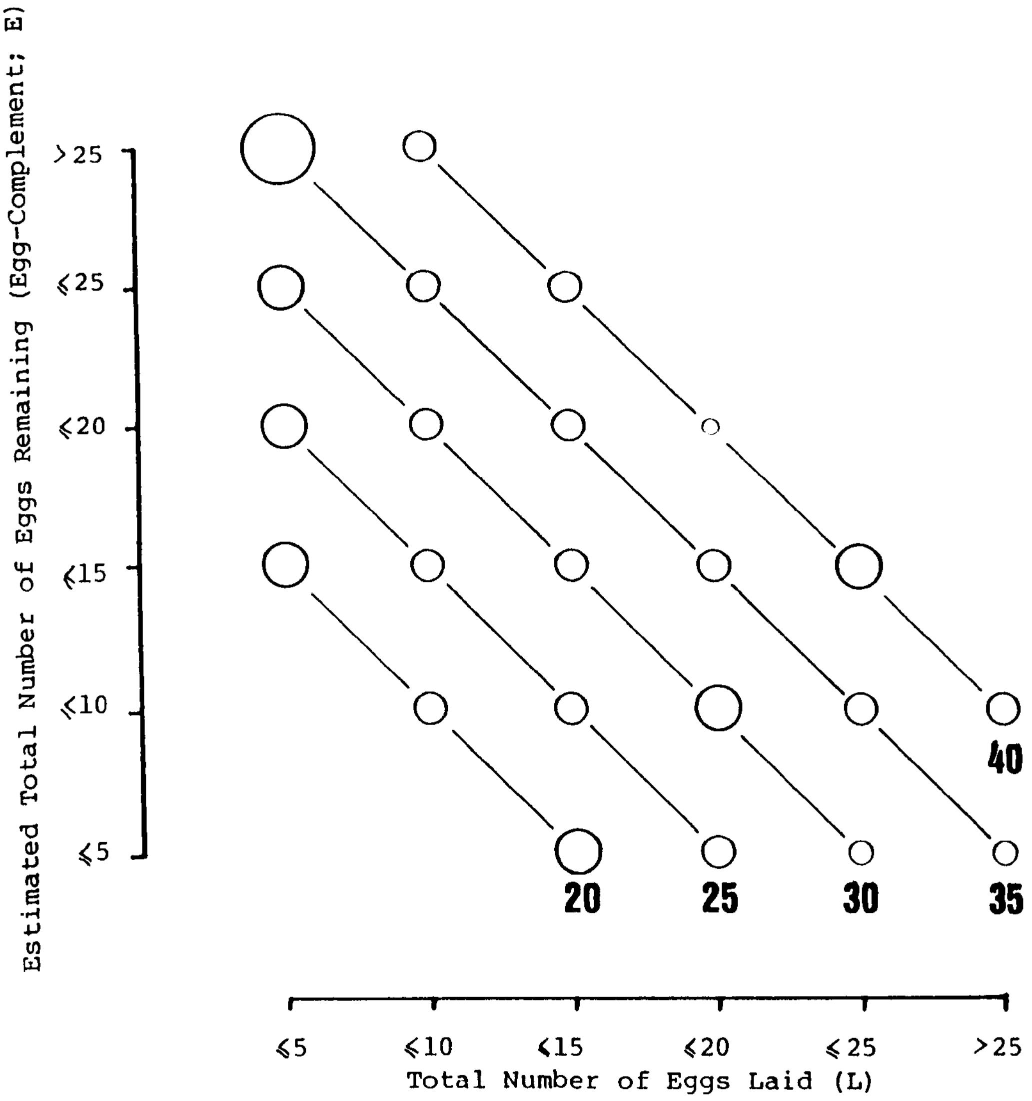


Figure 6.10 Clutch Size as a Function of Estimated Number of Eggs Remaining in the Oviducts (Egg-Complement) and Total Number of Eggs Laid for Females Given Seeds Varying in Size.

Median clutch sizes are indicated by diameter of circles (and symbols are consistent with Figs. 6.7-6.9). The 5 sizes of circle represent clutch sizes of 1-5 eggs/seed. Diagonal lines join together combinations of E and L that add up to a given value (estimated egg-storing capacity). These values are shown as numbers in bold face.

explain a significant amount of additional variance in clutch size (models 6-9, Table 6.4; tests VIII-X, Table 6.5). This suggests that the oscillations in Experiment 4.5 was also a consequence of the mechanism for determining clutch size.

Summary of General Linear Model Analysis.

(1) Seed value (egg-load or seed weight) explained a small, but significant amount of the variance in clutch size (7% and 3%, respectively).

(2) All physiological variables considered (current egg-complement, number of eggs laid and initial egg-complement) explained significantly more of the variance in clutch size.

(3) Inclusion of the female's current egg-complement in the model boosted the explainable variance up to 24% and 12%, respectively.

(4) Addition to the previous model of the total number of eggs laid by the female and interactions between the number of eggs laid and current egg-complement, increased the explained variance considerably (to 35% and 22%, respectively). This suggests that clutch size is determined by the female's current egg-complement relative to her ESC.

(5) The significance of the interaction terms in this model suggests that the relationshipbetween clutch size and relative egg-complement may not be a simple linear one.

(6) After these factors have been included in the model it is unnecessary to invoke an additional variable to explain the observed oscillations in clutch size. In other words, 'memory' of previous seed visits depends on physiological variables associated with oviposition and not with a special neurological mechanism.

DISCUSSION.

Clutch size variation, both within and between female C. maculatus, is considerable. In Chapter 4, seed value (egg-load and weight) was implicated as a major determinant of clutch size, but there was still a considerable amount of unexplained variation. Results from the present chapter strongly suggest that the internal state of the ovipositing female may explain much of this variation. Of the three internal state variables examined (egg-complement, number of eggs laid and relative egg-complement), the relative egg-complement of the ovipositing female explained most variation in clutch size.

In earlier experiments, clutch size was sensitive to host encounter rate, female age, female body size and previous laying experience, as well as to the egg-load and weight of the current seed. Computer simulations in which clutch size was determined solely by the interaction between seed value and relative egg-complement suggest that all of these trends could be accounted for by these two factors alone. Moreover, quantitative tests of the model showed that up to 35% of the variance in clutch size could be attributed to seed value and relative egg-complement. Clearly, clutch size determination is more complicated than these analyses would suggest (65% of clutch size variation in Expt. 4.2 was unexplained), but the essential elements of the decision-making process appear to be included in these two factors.

The current mechanistic model has several advantages over the previous (OUI) model, developed in Chapter 4:

(1) It predicts the observed temporal variation in clutch size.

(2) It predicts the observed variation between females.

(3) It obviates the need to invoke a detailed memory of previous host encounters or a sophisticated biological clock.

Generality of the Mechanism.

Biologists have recognised for many years that the motivational state of an animal is influenced by both internal and external factors: Lorenz's (1950) famous hydraulic model of motivation was based on interactions between these factors. However, there have been few good quantitative descriptions of how internal and external causal factors interact to produce particular behavioural tendencies (but see examples in McFarland & Houston 1981). Qualitative support for the importance of these interactions to insect oviposition behaviour is given by the study of Simbolotti *et al.* (1987), which indicates that the oviposition decisions of *Lariophagus* wasps are strongly influenced by the interaction between host size and egg-complement. Studies currently in progress may ultimately provide quantitative support for this view (Simbolotti *et al.* 1987).

Mechanistic models are necessarily less general in their applicability than functional models, but a mechanism based on the interaction between host value and egg-complement would appear to be relevant to all insects that are selected to maximise their rate of offspring production. Wasps and beetles are only distantly related, yet *Lariophagus* and *Callosobruchus* appear to share a common mechanism for clutch size determination. A thorough examination of oviposition behaviour in other insect orders would reveal how common this mechanism is within the class as a whole, and might also shed some light on its origin.

CHAPTER 7

GENERAL DISCUSSION.

Chapter 7. General Discussion.

The aim of the present study was to formulate and test functional and mechanistic models for clutch size variation in the bruchid Callosobruchus maculatus, and so identify the key selection pressures acting on oviposition behaviour in this species.

Six basic functional models were constructed (the Single Oviposition models), which differed from each other in the main constraint on clutch size. Experiments in which female encounter rate with hosts was altered gave qualitative support for four of these models, but the quantitative fit of all of them was poor. When the (*a priori*) condition was included in these models that several other females would also oviposit on the same host (the Multiple Oviposition models), the Time Limiting Multiple Oviposition (TLMO) model alone produced predictions that were quantitatively supported by experiments. For travel times ranging between 1 minute to 4 days, and for egg-loads ranging between 1 and 5 eggs/seed, the TLMO model predicted clutch sizes similar to those observed.

An important, if not totally unexpected, result from the present study is that the total number of eggs available to the female and the number of hosts that she can visit during her lifetime are not, by themselves, important constraints on clutch size: eggs-limiting and hosts-limiting models predict that females will be unresponsive to travel time and will lay clutch sizes of 1 and 15 eggs/seed, respectively. Because observed clutch sizes ranged between these values and increased with travel time, both models can be rejected. However, one can not unequivocably reject the remaining three models.

The reason why the eggs & time and eggs & hosts models cannot be rejected out of hand is that as well as predicting, in qualitative terms, the observed response to host encounter rate, both models also predict the observed temporal patterns in clutch size with respect to maternal phenotype. These patterns are not predicted by the TLMO model. Reluctance to reject the reserves-limiting model is based on the observed phenotypic trade-off between realised fecundity and lifespan (Fig. 3.5). Given this very clear result, it is paradoxical that reserves should not be implicated as a major constraint on clutch size. On its own, a reserves constraint could not explain the observed temporal decline in clutch size or the patterns associated with female body size. Therefore, it is likely that if reserves are limiting then additional constraints are also involved.

Physiological constraints on clutch size include the rate of egg maturation (Ch.5). _C. maculatus_ females mature eggs at a relatively slow rate: approximately one Lack clutch size (15 eggs) per day. This means that egg-limitation is likely to be a severe constraint on clutch size if seeds are encountered at a high rate, but less so as host encounter rates decline. (Note that the eggs & time and eggs & hosts models failed at high host encounter rates even when egg-limitation is taken into account by including low values for **T/E**; Table 3.3).

Price (1972) argued that the rate of egg maturation would evolve to match that of the usual encounter rate with hosts. It is unlikely that there is a 'usual' host encounter rate for stored product beetles, but if there is then, according to Price's hypothesis, for C. maculatus this is a maximum of approximately 15 pristine seeds/day. Ouedraogo & Huignard (1981) noted that the egg maturation rate of females that had been in culture for just a few generations was up to twice as high as that recorded in the present study. The difference between the two studies could be explained if the culturing regime employed during the present study had inadvertently relaxed selection for high maturation rates that usually operates in the field. In this respect it would be interesting to see whether, and how quickly, this 'constraint' responds to positive selection.

Egg maturation rate of individual females is not fixed, but can be lowered if hosts are rarely encountered (Ch.5). The observed flexibility in this response suggests that the frequency of host encounters varies within generations. It would be interesting to know whether different genotypes vary in the degree of flexibility in this response.

The problem of interpreting behaviours that are not responsive to prevailing conditions has already been discussed (Ch.3), but it is worth mentioning again because it has particular bearing on the present study. The response of female C. maculatus to future levels of oviposition appears to be fixed, whereas that to present levels of multiple oviposition (as indicated by current egg-loads), is flexible. A fixed response to a potential cue to habitat quality, such as the number of ovipositing females occupying it (i), may be due to one of several causes: (a) the cue is an unreliable indicator of habitat quality and is therefore not used; (b) the cue is reliable but the animal is incapable of reliably perceiving its true value; (c) habitat quality, as indicated by the cue, is unimportant to fitness and therefore there is no benefit in responding to the cue; and (d) the cue has not been correctly identified by the experimenter. Implication of the TLMO model suggests that females do not repond to the number of ovipositing females because of cause (a), i.e. that this is an unreliable indicator of the number of females that will subsequently lay on each seed. However, cause (d) cannot be excluded. Thorough testing of a model is impossible when the animal's response is independent of prevailing conditions, however a consistent response in a variety of situations may lend it some support. For example, the same value for i was obtained over the range of travel times and egg-loads used in the present study.

Although the models described in Chapter 2 were useful in excluding some possible constraints on clutch size and identifying others, the models were, nonetheless, very simple static, deterministic models. In other words, the decisions it predicted as being optimal were independent of all previous decisions and of stochasticity in the model parameters. The limitations of such models in making precise, quantitative predictions about fine-scale decisions has recently been highlighted by Houston et al. (1988). An interesting extension of the present study would be to include these same constraints in stochastic dynamic programming (SDP) models (McNamara & Houston 1986, Mangel & Clark 1986, Houston et al. 1988).

Clutch size decisions are particularly amenable to the dynamic programming approach because after each oviposition the internal state of the female (e.g. the number of mature and immature eggs in the oviducts) has changed and hence, if eggs or reserves are limiting, the optimal size of the next clutch must also have changed. Mangel (1987a, b, 1989) has recently applied dynamic programming models to the oviposition behaviour of several insect species and these have helped to distinguish between constraints that would be difficult to tell apart using conventional static models.

As well as having its own inherent value, a knowledge of the mechanisms involved in decision-making processes may also lend useful insights into the functional basis of behaviour. Computer simulations of several mechanistic models suggest that internal state-variables may play a vital role in the clutch size decisions of C. maculatus. The most important state-variable appears to be the number of mature eggs in the oviducts relative to the egg-storing capacity of the female. Analyses using General Linear Models indicate that up to thirty-five per cent of the variance in clutch size may be accounted for by this state-variable. A mechanism of this sort would be expected to evolve if the number of mature eggs available was the major constraint on clutch size. However, as the number of mature eggs in the oviducts is also a function of female age and previous oviposition experience, the dismissal of time, hosts or reserves as important constraints on behaviour would be premature until appropriate dynamic models have been tested.

There is now a wealth of qualitative support for various clutch size models, however, there is very little quantitative support for any of them (Godfray 1987). One of the reasons for this is that the assumptions of the models are often violated. Throughout the present study, emphasis has been placed on recognising and verifying the assumptions of the models being tested and on detailing a priori as many alternative models as possible, in order to reduce the need to make ad hoc predictions. Although a single model was implicated by the present study, for the reasons outlined above it is important to emphasise the Popperian view that a hypothesis can never be proved, only disproved.

The present study highlights one further point, and that is that behaviour which on the surface appears very complex, can in fact be controlled by very simple mechanisms. Clutch size decisions in *C. maculatus* are strongly influenced by previous actions. Whilst this may give the impression of a mechanism that involves memory and learning, the results of the Chapter 6 suggest that no such mechanism is required; decisions based entirely on the females current internal state and perception of her immediate surroundings will produce similar clutch size distributions to those observed. A close examination of the sorts of situations in which this kind of mechanism succeeds or fails to approximate the optimal solution may lend some useful insights into the main selection pressures acting on the oviposition behaviour of *Callosobruchus* beetles.

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APPENDIX 1

CLUTCH SIZE MODELS:

PROOF OF OPTIMAL SOLUTIONS UNDER VARIOUS ASSUMPTIONS.

Appendix 1. Clutch Size Models:

Proof of optimal clutch sizes under various assumptions.

Appendix 1.1. Hosts Limiting.

Model 1. Hosts limiting; single oviposition.

Fitness of clutch, $G(N) = N.s(N)$ [A1.1.1]

where N is clutch size, and $s(N)$ is the fitness of each egg as a function of clutch size. Therefore,

$dG(N)/dN = s(N) + N.s'(N)$ [A1.1.2]

where $s'(N)$ is the first derivative of $s(N)$ with respect to N.

When hosts are limiting, $N^{\wedge}$, the optimal clutch size, is that at which fitness gain from each host is at a maximum i.e. when, $dG(N)/dN = 0$ or:

$0 = s(N^{\wedge}) + N^{\wedge}.s'(N^{\wedge})$ [A1.1.3]

(i) Linear fitness function: $s(N) = a-bN,$ [A1.1.4]

$N^{\wedge}$ is found by substituting Eqn. A1.1.4, and its first derivative with respect to N, into Eqn. A1.1.3:

$0 = a-bN^{\wedge} - bN^{\wedge}$ [A1.1.5]

$= a-2bN^{\wedge}$ [A1.1.6]

Therefore,

$N^{\wedge} = a/2b$ [A1.1.7]

$= N^{*}$ [A1.1.8]

where N^{*} is the most productive clutch size.

(ii) Exponential fitness function: $s(N) = e^{-cN}$ [A1.1.9]

$\hat{N}$ is found by substituting Eqn A1.1.9, and its first derivative with respect to N, into Eqn A1.1.3:

$$0 = e^{-c\hat{N}} - c\hat{N}e^{-c\hat{N}} \quad [A1.1.10]$$

$$= (1-c\hat{N}).e^{-c\hat{N}} \quad [A1.1.11]$$

Because the right-hand side of Eqn. A1.1.11 (outside parentheses) is always greater than zero,

$0 = 1-cN$, and

$$\hat{N} = 1/c = N^* \quad [A1.1.12]$$

(iii) Non-linear convex fitness function: $s(N) = 1-cN^2$ [A1.1.13]

$\hat{N}$ is found by substituting Eqn. A1.1.13, and its first derivative with respect to N, into A1.1.3:

$$0 = 1 - c\hat{N}^2 - 2c\hat{N}^2 \quad [A1.1.14]$$

$$= 1 - 3c\hat{N}^2 \quad [A1.1.15]$$

therefore,

$$\hat{N} = (1/3c)^{\frac{1}{2}} \quad [A1.1.16]$$

Model 7(a). Hosts limiting, multiple oviposition I. (i.e. the current egg-load of all hosts is x eggs/seed).

Substitute $s(N,x)$ and $s'(N,x)$ for $s(N)$ and $s'(N)$ in eqns. A1.1.1 and A1.1.2. When hosts are limiting,

$$0 = s(\hat{N},x) + \hat{N}.s'(\hat{N},x) \quad [A1.1.17]$$

where $s'(N,x)$ is the first derivative of $s(N,x)$ with respect to N. Substitute $(N+x)$ for N in eqns. A1.1.4-A1.1.16:

(i) Linear fitness function. $s(N,x) = a-b(N+x)$ [A1.1.18]

$N^{\wedge}$ is found by substituting Eqn. A1.1.18, and its derivative, into Eqn. A1.1.17:

$$0 = a - b(N^{\wedge}+x) - bN^{\wedge} \quad [A1.1.19]$$

$$= a - 2bN^{\wedge} - bx \quad [A1.1.20]$$

therefore,

$$N^{\wedge} = (a-bx)/2b \quad [A1.1.21]$$

$$= a/2b - x/2 \quad [A1.1.22]$$

$$= N^{*} - x/2 \quad [A1.1.23]$$

where N^{*} is the most productive clutch size when the seed is egg-free (Eqn. A1.1.8).

xmax (maximum egg-load expected per seed) is found by setting $N^{\wedge}$ to zero and solving for x.

$$x_{max} = 2N^{*} \quad [A1.1.24]$$

(ii) Exponential fitness function: $s(N,x) = e^{-c(N+x)}$ [A1.1.25]

$N^{\wedge}$ is found by substituting Eqn. A1.1.25, and its derivative, into Eqn. A1.1.17:

$$0 = e^{-c(N^{\wedge}+x)} - cN^{\wedge}e^{-c(N^{\wedge}+x)} \quad [A1.1.26]$$

$$= (1-cN^{\wedge}).e^{-c(N^{\wedge}+x)} \quad [A1.1.27]$$

Because the right-hand side of Eqn. A1.1.27 (outside parentheses) is always greater than zero,

$$N^{\wedge} = 1/c = N^{*} \quad [A1.1.28]$$

i.e. when the fitness function is exponential, the optimal clutch size is independent of current egg-load of the host, and therefore there is no upper limit to x.

(iii) Non-linear convex fitness function: $s(N,x) = 1-c(N+x)^2$ [A1.1.29]

Substituting Eqn. A1.1.29, and its derivative, into Eqn. A1.1.17:

$0 = 1 - c\hat{N}^2 - cx^2 - 2c\hat{N}x - 2c\hat{N}^2 - 2c\hat{N}x$ [A1.1.30]

$= 1 - c(3\hat{N}^2 + 4x\hat{N} + x^2)$ [A1.1.31]

dividing throughout by 3c,

$0 = 1/3c - (1/3)(3\hat{N}^2 + 4x\hat{N} + x^2)$ [A1.1.32]

$= \hat{N}^2 + 4/3.x\hat{N} + 1/3.x^2 - N^{*2}$ [A1.1.33]

solving the quadratic for $\hat{N}$ and ignoring negative root,

$\hat{N} = -2/3.x + [(N^{*2} - 2/9.x^2)^{\frac{1}{2}}]$ [A1.1.34]

and,

$xmax = 3^{\frac{1}{2}}N^*$ [A1.1.35]

Model 7(b). Hosts limiting; multiple oviposition II. (i.e. the total number of females laying on each host is equal to i).

Substitute $s(N,i)$ and $s'(N,i)$ for $s(N)$ and $s'(N)$ in eqns. A1.1.1 and A1.1.2. When hosts are limiting,

$0 = s(\hat{N},i) + \hat{N}s'(\hat{N},i)$ [A1.1.36]

Substitute $(i-1)$ for x in eqns. A1.1.18-A1.1.35:

(i) Linear fitness function. $s(N,i) = a-b[N+(i-1)N]$ [A1.1.37]

$= a-biN$ [A1.1.38]

Substitute Eqn. A1.1.38, and its first derivative with respect to N, into Eqn. A1.1.36:

$0 = a - bi\hat{N} - bi\hat{N}$ [A1.1.39]

$= a - 2biN^{\wedge}$ [A1.1.40]

therefore,

$N^{\wedge} = a/2bi$ [A1.1.41]

$= N^{*}/i$ [A1.1.42]

xmax is found by multiplying $N^{\wedge}$ by i:

$xmax = N^{*}$ [A1.1.43]

(ii) <u>Exponential fitness function.</u> $s(N,i) = e^{-ciN}$ [A1.1.44]

Substitute Eqn. A1.1.44, and its derivative, into Eqn A.1.1.36:

$0 = e^{-ciN^{\wedge}} - ciN^{\wedge}e^{-ciN^{\wedge}}$ [A1.1.45]

$= (1-ciN^{\wedge})e^{-ciN^{\wedge}}$ [A1.1.46]

Because the right-hand side of eqn. A1.1.45 (outside parentheses) is always greater than zero,

$0 = (1-ciN^{\wedge})$, and

$N^{\wedge} = 1/ci$ [A1.1.47]

$= N^{*}/i$ [A1.1.48]

and,

$xmax = N^{*}$ [A1.1.49]

(iii) <u>Non-linear convex fitness function</u>: $s(N,i) = 1-ci^{2}N^{2}$ [A1.1.50]

Substituting Eqn. A1.1.50, and its derivative, into Eqn. A1.1.36:

$0 = 1 - ci^2\hat{N}^2 - 2ci^2\hat{N}^2$ [A1.1.51]

$\quad = 1 - 3ci^2\hat{N}^2$ [A1.1.52]

therefore,

$\hat{N} = (1/3ci^2)^{\frac{1}{2}}$

$\quad = N^*/i$ [A1.1.54]

and,

$xmax = N^*$ [A1.1.55]

Appendix 1.2. Time Limiting.

Model 2. Time limiting; single oviposition.

Rate of fitness gain, $F(N) = N.s(N)/(Tt+NTo)$ [A1.2.1]

where N is clutch size, $s(N)$ is the fitness of each egg as a function of clutch size, Tt is travel time, and To is oviposition time.

Therefore, rate of fitness gain is maximised when $dF/dN = 0$

i.e. when

$$0 = [(Tt+\hat{N}To)(s(N)+Ns'(N))-(\hat{N}s(\hat{N})To)] \;/\; [(Tt+\hat{N}To)\;] \qquad [A1.2.2]$$

$$= [(Tt+\hat{N}To)(s(\hat{N})/s'(\hat{N}) + \hat{N})] - [\hat{N}s(\hat{N})/s'(\hat{N})To] \qquad [A1.2.3]$$

$$= [s(\hat{N})/s'(\hat{N})][1 - \hat{N}To/(Tt+\hat{N}To)] + \hat{N} \qquad [A1.2.4]$$

Therefore,

$$\hat{N} = [-s(\hat{N})/s'(\hat{N})][Tt/(Tt+\hat{N}To)] \qquad [A1.2.5]$$

(i) Linear fitness function. $s(N) = a-bN$ [A1.1.4]

Substituting Eqn. A1.1.4 into Eqn. A1.2.5:

$$\hat{N} = [(-a+b\hat{N})/-b][Tt/(Tt+\hat{N}To)] \qquad [A1.2.6]$$

$$= [2N^*-\hat{N}][Tt/(Tt+\hat{N}To)] \qquad [A1.2.7]$$

$$0 = To\hat{N}^2 + 2Tt\hat{N} - 2N^*Tt \qquad [A1.2.8]$$

Solving the quadratic for $\hat{N}$ and ignoring negative root,

$$\hat{N} = [-Tt + (Tt^2 + ToTt2N^*)^{1/2}] \;/\; To \qquad [A1.2.9]$$

(ii) Exponential fitness function: $s(N) = e^{-cN}$ [A1.1.9]

Substituting Eqn. A1.1.9 into Eqn A1.2.5:

$$\hat{N} = [e^{-c\hat{N}}/-ce^{-c\hat{N}}][Tt/(Tt+\hat{N}To)] \qquad [A1.2.10]$$

$$= (1/c)[Tt/(Tt+\hat{N}To)] \qquad [A1.2.11]$$

$$= (TtN^*)/(Tt+\hat{N}To) \qquad [A1.2.12]$$

therefore,

$$0 = To\hat{N}^2 + Tt\hat{N} - TtN^* \qquad [A1.2.13]$$

Solving for $\hat{N}$ and ignoring negative root,

$$\hat{N} = [-Tt + (Tt^2 + ToTt4N^*)^{\frac{1}{2}}] / 2To \qquad [A1.2.14]$$

Model 8(a). Time limiting; multiple oviposition I (current egg-load of host is x eggs/seed).

Substitute $s(N,x)$ and $s'(N,x)$ for $s(N)$ and $s'(N)$ in Eqn. A1.2.5.

$$\hat{N} = [-s(\hat{N},x)/s'(\hat{N},x)][Tt/(Tt+\hat{N}To)] \qquad [A1.2.15]$$

(i) Linear fitness function. $s(N,x) = a-b(N+x)$ [A1.1.18]

Substituting Eqn. A1.1.18 and its derivative into Eqn. A1.2.15:

$$\hat{N} = [(-a+b(\hat{N}+x))/-b][Tt/(Tt+\hat{N}To)] \qquad [A1.2.16]$$

$$= [2N^*-\hat{N}-x][Tt/(Tt+\hat{N}To)] \qquad [A1.2.17]$$

Therefore,

$$0 = To\hat{N}^2 + 2Tt\hat{N} - (2N^*-x)Tt \qquad [A1.2.18]$$

solving the quadratic for $\hat{N}$ and ignoring negative root,

$$\hat{N} = [-Tt + (Tt^2 + ToTt(2N^*-x))^{\frac{1}{2}}] / To \qquad [A1.2.19]$$

and,

$$xmax = 2N^* \qquad [A1.2.20]$$

(ii) Exponential fitness function. $s(N,x) = e^{-c(N+x)}$ [A1.1.25]

Substituting Eqn. A1.1.25 and its derivative into Eqn A1.2.15:

(ii) Exponential fitness function: $s(N,i) = e^{-ciN}$ [A1.1.44]

Substituting Eqn. A1.1.44 and its derivative into Eqn A1.2.26:

$N^{\wedge} = [-e^{-ciN^{\wedge}}/-cie^{-ciN^{\wedge}}][Tt/(Tt+N^{\wedge}To)]$ [A1.2.32]

$= (N^*/i)[Tt/(Tt+N^{\wedge}To)]$ [A1.2.33]

Therefore,

$0 = To/TtN^{\wedge 2} + N^{\wedge} - N^*/i$ [A1.2.34]

Solving for $N^{\wedge}$ and ignoring negative root,

$N^{\wedge} = [-Tt + (Tt^2 + ToTt4N^*/i)^{\frac{1}{2}}] / 2To$ [A1.2.35]

and,

$xmax = [-iTt + (i^2Tt^2 + ToTt4N^*i)] / 2To$ [A1.2.36]

i.e. as i and Tt approach infinity, $xmax$ approaches N^*.

$$\hat{N} = [-e^{-c(\hat{N}+x)} / -ce^{-c(\hat{N}+x)}]\,[Tt/(Tt+\hat{N}To)] \qquad [A1.2.21]$$

$$= (1/c)\,[Tt/(Tt+\hat{N}To)] \qquad [A1.2.22]$$

$$= (N^*Tt)/(Tt+\hat{N}To) \qquad [A1.2.23]$$

Therefore,

$$0 = To\hat{N}^2 + Tt\hat{N} - N^*Tt \qquad [A1.2.24]$$

Solving quadratic for $\hat{N}$ and ignoring negative root,

$$\hat{N} = [-Tt + (Tt^2 + ToTt4N^*)^{\frac{1}{2}}] \,/\, 2To \qquad [A1.2.25]$$

i.e. $\hat{N}$ is unaffected by the presence of eggs on the host and there is therefore no upper limit to the number of eggs per seed, **xmax**.

Model 8(b). Time limiting; multiple oviposition II (number of ovipositing females is equal to i).

Substitute s(N,i) and s'(N,i) for s(N) and s'(N) in Eqn. A1.2.5:

$$\hat{N} = [-s(\hat{N},i)/s'(\hat{N},i)]\,[Tt/(Tt+\hat{N}To)] \qquad [A1.2.26]$$

(i) Linear fitness function. $s(N,i) = a-biN$ [A1.1.38]

Substituting Eqn. A1.1.38 into Eqn. A1.2.26:

$$\hat{N} = [(-a+bi\hat{N})/-bi]\,[Tt/(Tt+\hat{N}To)] \qquad [A1.2.27]$$

$$= [(2N^*/i)-\hat{N}]\,[Tt/(Tt+\hat{N}To)] \qquad [A1.2.28]$$

Therefore,

$$0 = To/Tt\hat{N}^2 + 2\hat{N} - (2N^*/i) \qquad [A1.2.29]$$

solving for $\hat{N}$ and ignoring negative root,

$$\hat{N} = [-Tt + (Tt^2 + ToTt2N^*/i)^{\frac{1}{2}}] \,/\, To \qquad [A1.2.30]$$

and,

$$xmax = [-iTt + ((i^2Tt^2 + TtTo2N^*i) \;] \,/\, To \qquad [A1.2.31]$$

i.e. As i or Tt approaches infinity, **xmax** approaches **N***.

Appendix 1.3. Eggs & Time Limiting.

Model 5. Eggs & time limiting; single oviposition.

Eggs limiting.

Eggs are the sole limiting resource when:

$T > T_E$ [A1.3.1]

where T = total time available (lifespan) and T_E = time required to lay all eggs.

$T_E = (NTo + Tt)@$ [A1.3.2]

where N = clutch size, To = oviposition time (and hence NTo = time taken to lay each clutch), Tt is travel time, and $@$ = number of clutches.

When eggs are limiting, $\hat{N}_E = 1$ (see model 3, Ch.2) and therefore,

$@_E = E$ [A1.3.3]

and, following Eqn. A1.3.2,

$T_E = (To + Tt)E$ [A1.3.4]

i.e. eggs are the sole limiting resource when:

$T > (To + Tt)E$ [A1.3.5]

or,

$T/E > (To + Tt)$ [A1.3.6]

Time limiting.

Time is the sole limiting resource when:

$T < T_E$ [A1.3.7]

When time is limiting, $\hat{N}_T = \tilde{N}$ (model 2, Ch.2) and therefore,

$\theta_T = E/\bar{N}$ [A1.3.8]

and, following Eqn. A1.3.2,

$T_E = (\bar{N}To + Tt)E/\bar{N}$ [A1.3.9]

$= (To + Tt/\bar{N})E$ [A1.3.10]

i.e. time is the sole limiting resource when:

$T < (To + Tt/\bar{N})E$ [A1.3.11]

or,

$T/E < (To + Tt/\bar{N})$ [A1.3.12]

where,

$\bar{N} = [-s(\bar{N})/s'(\bar{N})][Tt/(Tt + \bar{N}To)]$ [A1.2.5]

<u>Eggs & time limiting</u>.

Eggs and time are both limiting when:

$(To + Tt) < T/E < (To + Tt/\bar{N})$ [A1.3.13]

If it pays to use up all eggs and all time (as indicated by the monotonically increasing fitness gain curves, Fig. 2.3), then:

$\hat{\theta}_E = \hat{\theta}_T$ [A1.3.14]

i.e.

$E/\hat{N}_E = T/(Tt + \hat{N}_T To)$ [A1.3.15]

and,

$\hat{N}_{ET} = Tt/(T/E - To)$ [A1.3.16]

Note that $\hat{N}_{ET}$ is independent of the fitness function, s(N), and that the transition between $\hat{N}_E$ and $\hat{N}_T$ is non-linear with respect to T/E (see Fig. 2.3).

Model 11. Eggs & time limiting, multiple oviposition.

Appendix 1.2 derives $N^{\wedge}$ when *time* is the only limiting factor and egg-loads (x) are greater than zero or the number of ovipositing females (i) is greater than 1.

When *eggs* are the sole limiting factor, $N^{\wedge}$ is always equal to 1.

When *both* are eggs and time are limiting, $N^{\wedge}$ is independent of x or i, but x and i affect the lower threshold value of T/E (right-hand side of Eqn. A1.3.13) by influencing $N^{\sim}$ (see Fig. 2.3(b),(c)). Increasing x or i, reduces $N^{\sim}$ and hence raises the lower threshold, until the lower threshold coincides with the upper threshold.

Appendix 1.4. Eggs & Hosts Limiting.

Model 6. Eggs & hosts limiting; single oviposition.

Eggs limiting

When eggs are the sole limiting resource, $\hat{N}_E = 1$ (model 2, Ch.2). Eggs are limiting, therefore, when the number of eggs available (E) is less than the number of hosts (H). That is, when

$$E < H \qquad [A1.4.1]$$

or,

$$E/H < 1 \qquad [A1.4.2]$$

Hosts limiting

When hosts are the sole limiting resource, $\hat{N}_H = N^*$ (model 1, Ch.2). Hosts are limiting, therefore, when:

$$E > HN^* \qquad [A1.4.3]$$

or,

$$E/H > N^* \qquad [A1.4.4]$$

Eggs & hosts limiting.

Eggs and hosts are both limiting when:

$$1 < E/H < N^* \qquad [A1.4.5]$$

If it pays to use up all eggs and all hosts, then:

$$\hat{N}_{EH} = E/H \qquad [A1.4.6]$$

Note that $\hat{N}_{EH}$ is independent of the shape of the fitness function.

Model 12. Eggs & hosts limiting, multiple oviposition.

Appendix 1.1 derives $\hat{N}$ when the number of *hosts* is the only limiting factor and egg-loads (x) are greater than zero or the number of ovipositing females (i) is greater than 1.

When *eggs* are the sole limiting factor, $\hat{N}$ is always equal to 1.

When *both* eggs and hosts are limiting, $\hat{N}_{EH}$ is independent of x or i, but x and i affect the upper threshold value of E/H (right-hand side of Eqn. A1.4.5) by influencing N^* (see Fig. 2.4). Increasing x or i, reduces N^* and hence lowers the upper threshold, until the upper threshold coincides with the lower threshold and $\hat{N}$ is always equal to one.

APPENDIX 2

STEPWISE GENERAL LINEAR MODELLING PROCEDURES.

Appendix 2. Stepwise General Linear Modelling Procedures.

The General Linear Modelling (GLM) procedure of SAS (SAS Institute Inc. 1985) is a multivariate analysis of variance and covariance program that uses least squares methods to estimate a variety of statistics for many different types of linear model. GLM was used extensively in the present study to perform stepwise analyses of covariance when the dependent variable was observed to covary with more than one independent variable. For example, clutch size (the dependent variable) declined as a function of seed weight (the treatment) and time (the covariate; Ch.4). Therefore, in order to compare mean clutch sizes on seeds differing in weight, it was necessary to control for the effect of time. GLM allowed this to be done.

The analysis generally involves 3 steps: first, the degree of the model is determined (i.e. first or second degree polynomial); second, the significance of the interaction(s) between the covariate(s) and the treatment is ascertained; and third, the significance of the treatment is determined after controlling for the covariate(s). These procedures are now illustrated in detail using the example given above.

First, a 'primary' model is constructed that includes the following parameters: G (treatment), X and X^2 (covariates), and X*G and X^2*G (interactions between the covariates and the treatment). The covariate X^2 is included in the primary model because the relationship between X and Y may be non-linear (X^3 is not included in the model because the number of interaction terms then becomes unwieldy).

To determine whether the relationship between X and Y is linear or quadratic, the primary model (model 1, Table A2.1(a)) is compared with the equivalent linear model (model 2, Table A2.1(a)). The statistical significance of the difference in the amounts of variance explained by the linear and quadratic models is determined by comparing the calculated F value with the appropriate tabulated value (test A, Table A2.1(b)). F is calculated as follows:

$$F = (SS_I - SS_{II})/(df_I - df_{II}) \ / \ MS_{I[error]}$$

with $(df_I - df_{II})$ and $df_{II[error]}$ degrees of freedom

where I and II refer to the two models being compared ($df_I > df_{II}$), SS is the model sum of squares, df is the degrees of freedom, and $MS_{I[error]}$ is the error mean square for model I (Draper & Smith 1966).

A significant F value indicates that the relationship between clutch size and time is quadratic, whereas a non-significant result indicates that either clutch size and time are linearly related or time is not a covariate after all. This could be determined by comparing model 2 with a model that does not include any covariates (model 3; test B,

(a)

MODEL	MODEL PARAMETERS
1	X X^2 G X*G X^2*G
2	X G X*G
3	G
4	X G
5	X
6	X X^2 G
7	X X^2

(b)

TEST	MODEL 1	MODEL 2	DIFFERENCE significant	 non-significant
A	1	2	quadratic (GOTO E)	linear (GOTO B)
B	2	3	X is covariate (GOTO C)	X not covariate (compare means)
C	2	4	significant interaction (STOP)	non-significant interaction (GOTO D)
D	4	5	treatments do differ (STOP)	treatments do not differ (STOP)
E	1	6	significant interaction (STOP)	non-significant interaction (GOTO F)
F	6	7	treatments do differ (STOP)	treatments do not differ (STOP)

Table A2.1 Models Used and Comparisons Performed in Step-wise Analysis of Covariance Using GLM.

See text for details of how to calculate significances. X = covariate, X^2 = covariate squared, G = treatment term.

Table A2.1(b)). A non-significant F value would mean that time was not a significant covariate and therefore that the treatments could be compared directly.

Assuming that time is a significant covariate, the next step is to determine the significance of the interaction terms in the model ($X*G$ and X^2*G). This is achieved by comparing equivalent models that include or exclude these terms. The precise comparison made depends on the outcome of the previous step. If the relationship between X and Y is quadratic, then model 1 is compared with model 6 (test E), and if it is linear then models 2 and 4 are compared (test C). The significance of the difference in explained variance is again determined by calculating an F value. If the model that includes the interaction terms explains significantly more of the variance in the dependent variable than the model that lacks them, then this implies that the relationship between the dependent variable and the covariate changes with the value of the treatment term. In this situation, nothing can be said about the relationship between the treatment and the dependent variable, and the analysis is complete. However, if there is no difference in the amount of explained variance between the two models, then the analysis can go on to the next step, which is to compare treatments.

The significance of the treatment term is determined by comparing equivalent models with and without the treatment term. Again, the exact comparison made depends on whether the model is linear or quadratic. The linear comparison is between models 4 and 5 (test D), the quadratic comparison between models 6 and 7 (test F). These tests indicate whether the treatment (e.g. seed weight) has a significant influence on the dependent variable (e.g. clutch size) after the effect of the covariate(s) (e.g. time) has been controlled for. At this point the analysis is complete.