CANNIBALISM IN A CHRYSOMELID BEETLE, *GASTROPHYSA VIRIDULA*

A THESIS

SUBMITTED IN ACCORDANCE WITH THE REQUIREMENTS OF

THE UNIVERSITY OF LIVERPOOL

FOR THE DEGREE OF DOCTOR IN PHILOSOPHY

by

Helen Margaret Kirk

December 1988

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I am especially grateful to my supervisor, Dr. Geoff Parker. He not only encouraged me to carry on when I most felt like giving up, but he also helped me to maintain an interest in what I was doing and to keep the many tedious hours of research in perspective. The enthusiastic way in which he shared with me his ideas on various aspects of the thesis provided the sort of stimulation that, at times, makes it all seem worthwhile. Perhaps his greatest expression of faith was in letting me borrow his computer at weekends - a very big sacrifice and much appreciated.

Dr. John Whittaker of Lancaster University introduced me to the main character of the thesis, the green dock leaf beetle *Gastrophysa viridula.* Without this introduction I am sure that I would not have developed such an interest in cannibalism and the title of the thesis would most likely have borne a closer resemblance to that originally intended - "Clutch Size in Invertebrates". So I am very grateful to Dr. Whittaker for this introduction and for his generosity in providing me with all the information and advice that I needed to have on the natural history of the beetle and how to rear it in the laboratory. I would also like to thank Joanne Ramsell and Peter Flint from Lancaster University. They came to the rescue on several occasions by helping me to find replacement beetles when things had gone wrong with my own laboratory stocks.

At Liverpool University I have many people to thank. Paul Ward and Leigh Simmons were extremely patient and good-humoured whenever they heard the familiar knock on their doors that usually meant "Can I ask you something?" I was always grateful for the way in which they seemed to be able to turn what appeared to me to be a horrible mess into something more orderly and comprehensible, especially where statistics were concerned. This help and advice was often given while they were earnestly peering over rows of dung flies in bottles, their heads bobbing this way and that so as not to miss anything vital. Their obvious dedication and interest in their subject was infectious - and sometimes entertaining!

Donna Fernandes helped me in many practical ways, particularly in the realm of anything to do with computers. But most of all she was both sympathetic and constructive whenever I had anything to moan about. It was reassuring to hear of her own experiences under similar circumstances and I am glad that she came to Liverpool to do her post-doctoral fellowship - I only wish she had come sooner.

Fiona Heron, Simon Gribbin and Sally Collins looked after the beetles when I was on holiday. This was a lot to ask of anyone since there were quite a lot of them and they were not always so easy to keep in the land of the living as might have been hoped for. So I am very grateful for having been given the opportunity to take a break from these creatures from time to time. Simon also showed extreme politeness in not rebuking me for the mess at my side of the room we shared in the Zoology department - even when it spread on occasions to take over most of the room. I did not realize the extent of his politeness until I saw him pruning his fuchsia to get rid of some aphids, which he told me he could not stand - and this was months after I myself had brought loads of aphid-infested dock plants to our room.

Eddie Jenion, Hazel Lewis and Ipek Kurtboke were very tolerant of an invasion of insects into the Botany department, especially Ipek who was used to nothing larger than actinomycetes in her laboratory. They all helped me to find my way around the facilities of their department and I learned a lot during my stay there. In particular, I would like to thank David Salt who helped to introduce me to the world of biochemistry by correcting my misconception that TLC and GLC were part of some strange language that I was unlikely ever to come to grips with. I would never have embarked on any biochemical analysis if it had not been for his encouragement and clear and patient explanations of things I had never even heard of, let alone understood. Steve Kershaw was very generous in offering to carry out part of the analysis of the oleic acid content of eggs using his own apparatus. I am grateful to him for that.

I am also grateful to Dr. Tom Gilchrist in the department of Organic

Chemistry for showing me how to carry out some of the techniques necessary for biochemical analysis of the defensive chemicals in the beetle eggs. Professor Jaques Pasteels of the Universite Libre de Bruxelles was more than generous in inviting me to visit his laboratory to learn the technique of "milking" beetles for their defensive secretions.

At Liverpool University's Botanical Gardens I would like to thank Mr. Ken Hulme and his staff, especially Paul Mathews. I know that dock plants were not their favourite botanical specimens in the gardens, but they did not complain too loudly about having to put up with such eye-sores. I was given a lot of help in rearing the plants and in setting up a field experiment. Sally Collins, Alexa Smith and Simon Gribbin also helped to set up this experiment.

I am very grateful to Dr. Jim Shaw for generously giving up some of his own time to carry out the irradiation of male beetles for the sperm competition experiment. Pat Owen was kind enough to arrange to have the thesis printed out in her department and I am grateful to her for that.

Finally, I would like to thank Pascal for the plums. Even if I had to shake the tree very hard sometimes to get them, they were sweet in the end and worth waiting for.

CANNIBALISM IN A CHRYSOMELID BEETLE, *GASTROPHYSA VIRIDULA*

Helen Margaret Kirk

ABSTRACT

Cannibalism is a very common phenomenon throughout the animal kingdom. Females of the green dock leaf beetle, *Gastrophysa viridula*, lay clutches of eggs on the leaves of dock plants (*Rumex* species). Cannibalism takes the form of larvae eating unhatched conspecific eggs. Both within-clutch and inter-clutch cannibalism were observed. Within-clutch cannibalism was found to be of very limited extent. However, two observations indicated that cannibalism in *G. viridula* is more important than this finding might suggest. First instar larvae proved to be much more voracious cannibals when given an unlimited supply of newly laid eggs than when in normally hatching clutches. Also, viable as well as non-viable eggs were eaten from within clutches.

The individual fitness benefits gained through cannibalism were studied. Larvae fed on eggs alone were unable to complete the first instar. But larvae given young eggs during their first instar along with dock leaves throughout all three instars did better in terms of survival and developmental rates than larvae fed only on dock leaves. No relationship was found between adult female size and lifetime egg production or longevity. Females were found to lay consecutively larger and smaller clutches throughout most of their lives.

Mathematical models were used to interpret the results of a sperm competition experiment. The results were consistent with two mechanisms of sperm competition: linear transfer of sperm with time from male to female and sperm removal or repositioning with sperm mixing, or diminishing transfer of sperm with time and sperm mixing without displacement. The individual benefits of cannibalism in terms of survival observed in the laboratory were sufficient to satisfy Hamilton's inclusive fitness criterion for the evolution of selfish behaviour, even for the case of larvae eating full-siblings.

It was anticipated that mechanisms may have evolved to reduce the risks of inter-clutch cannibalism. The distance from previously laid clutches that females lay their eggs was found to be important in this respect, and an intrinsic protective mechanism against cannibalism which becomes more effective with age was found. A possible link between this mechanism and chemical defence normally assumed to have evolved as a mechanism against inter-specific predation is suggested. It was found that the relative concentration of total oleic acid increased with age in *G. viridula* eggs, whereas the relative concentrations of all other major fatty acids declined or remained constant. The possible anti-cannibalistic role of oleic acid merits further investigation.

The relationships between clutch size, asynchrony of hatching and the extent of within-clutch cannibalism were also studied. Asynchrony of hatching was found to increase with clutch size. Although negative relationships were found between hatching success of viable eggs and both clutch size and asynchrony of hatching, multiple regression showed that clutch size was the primary factor affecting the hatching success rate of viable eggs.

An ability to distinguish between related and non-related eggs as victims was not found among first instar larvae. This finding is discussed in the light of individual and kin selection and the probability of encountering related eggs.

CHAPTER 1

INTRODUCTION

The aim of this thesis is to investigate various aspects of cannibalism in a species of insect which lays clutches of eggs on its host plant.

Gastrophysa viridula

The animal subject around which this thesis centres is a chrysomelid beetle, *Gastrophysa viridula* Degeer, or the green dock leaf beetle. The chrysomelids constitute one of the major families of Coleoptera. These insects are mostly phytophagous and include some well known agricultural pests such as the Colorado beetle, *Leptinotarsa decemlineata*, a notorious pest of potatoes. Two species, *Chrysolina brunsvicensis* and *C. quadrigemina* have been used to control the Klamath weed, *Hypericum perforatum* (Holloway, 1964).

As the name suggests, the green dock leaf beetle has green iridescent elytra and pronotum in the adult form which is about 3mm. long. It is an oligophagous herbivore and its main host plants are the broad-leaved dock (*Rumex obtusifolius* L.) and the curly dock (*Rumex crispus* L.). All the active stages feed on dock leaves and the beetles are found largely in unstable habitats such as roadside verges, waste land, meadows in which the host plants are periodically cut and river and shingle banks.

Gastrophysa viridula spends the autumn, winter and early spring as the adult stage in the soil surrounding its host plants. The diapause is broken in May when males and gravid females can be found on the dock plants. The females, in common with many other chrysomelids (Klausnitzer and Forster, 1971), lay single-layer clutches of bright yellow eggs on the under-surfaces of the leaves of their host plants. Smith and Whittaker (1980a) reported that females are able to lay up to 1000 eggs in clumps with a mean egg number of 25-35 depending on the habitat. Whittaker *et al.* (1979) reported that the maximum number of eggs laid by one female in the laboratory was 1377 in 38 clutches. When active, the adults can survive in the field for up to two months (Smith and Whittaker, 1980a). The larvae are black and take 3-4 weeks to pass through 3 instars on the dock plants. The third instar larvae enter the soil surrounding the plants and there metamorphose into bright yellow pupae. Emerging adults then climb back onto the plants. A generation may be completed in 4-6 weeks and it is possible for 3 generations to occur in a year in the north-west of England.

The details of the life history of this beetle were obtained from personal observations during a large-scale field experiment and from some of the publications of Dr. John Whittaker and his colleagues at Lancaster University (e.g. Whittaker *et al.*, 1979; Smith and Whittaker, 1980a,b). A fuller description of the experiment in which some of these observations were made will be given in the General Discussion. The aim of the experiment was to investigate the relationship between the number of eggs laid per unit area of dock leaf and the subsequent development and survival of the resulting larvae. The number of eggs laid on each of similar-sized plants was controlled at the outset. But that summer, the summer of 1985, was a particularly wet one and there were many slugs around. These slugs badly interfered with the numbers of eggs on each plant. On most plants the numbers of eggs were severely reduced. So that particular line of research was abandonned, but the experiment was not altogether a lost cause. It was during that time that I discovered that newly hatched larvae of *G. viridula* will eat unhatched eggs from within their own clutches. Cannibalism subsequently became the main theme of the research carried out for this thesis.

The green dock leaf beetle has been the subject of an extensive programme of research at Lancaster University. An examination of the published papers arising from this research allows a useful summary of the current knowledge of the biology of *G. viridula* to be made before proceeding in succeeding chapters to investigate more specifically the cannibalism found in this species.

Whittaker *et al.* (1979) and Smith and Whittaker (1980b) have listed some of the common predators of *Gastrophysa viridula.* They have reported that, particularly in the first generation, egg mortality may occur as a result of syrphid eggs being laid in the midst of a *G. viridula* egg clutch. For example, Whittaker *et al.* (1979) reported that up to 50% of the mortality in the first generation of a population of *G. viridula* on a river bank, near Lancaster, was attributable to syrphid larvae feeding on eggs or newly hatched first instars. The syrphid eggs are white and so are easily seen amongst the bright yellow *G. viridula* eggs. Any clutches containing a syrphid egg and any others on the same leaf or even on adjacent parts of the plant are apparently sure to be eaten when the syrphid hatches. A single syrphid larva may consume up to 200 eggs and larvae in the course of its development.

Heteropteran predators were also found, *Anthocoris nemorum* L. being the most common. *A. nemorum* adults and penultimate instar nymphs feed on the eggs and all the larval instars of *G. viridula.* Spiders were found to cause high rates of mortality among adult *G. viridula*, particularly of the second generation. The most abundant spider found was an unidentified web spinner. On a rotivated area of land in which docks were planted and other species of weeds allowed to grow back, Smith and Whittaker (1980b) estimated that 75% of the peak number of second generation adults were predated by spiders. Whittaker *et al.* (1979) reported that rates of parasitism on *G. viridula* were extremely low.

Adult *Gastrophysa viridula* disperse by walking. They have never been observed flying either in the field or when reared in the laboratory (Smith and Whittaker 1980b). Other workers have noted that *Gastrophysa* species are poor dispersers (Zwoelfer, 1973; Potts and Vickerman, 1974; Whittaker *et al.* 1979). These animals therefore rely on walking as their main method of large scale population movement.

Smith and Whittaker (1980b) studied the dispersal behaviour of adult *G. viridula.* Five hundred adult beetles were released into the centre of a diverse vegetaion plot containing *R. obtusifolius* plants. The site was divided into a notional central circle of radius 1m. and seven concentric annuli, each with a width of 1m. The numbers of beetles found on the plants in each annulus were recorded on eight successive days. Dispersal resulted in a redistribution of the beetles roughly in proportion to the number of plants in each annulus, rather than emigration from the plot.

The same authors investigated how efficiently the adult beetles could locate new plants once they were within a clump of suitable hosts. They determined the degree of mixing which occurred between animals found on plants in a plot which were originally marked, and the population of animals that were moving between the plants which were originally unmarked. They found that although there was extensive population movement between the plants on the plot, each beetle was, on average, able to locate a new plant in one day. The authors concluded that it is unlikely that such a short delay in locating new food sources and oviposition sites could have any serious effects on population mortality.

Overall, they concluded that adult beetles, whilst moving a good deal between the plants within a plot, may be comparatively unwilling to leave a clump and search for feeding sites elsewhere. They therefore considered that large scale population movement over distances greater than 40-50 metres is unlikely. Whittaker *et al.* (1979) found that *G. viridula* adults recolonizing an area of shingle with *Rumex obtusifolius* plants did so at a rate of only approximately 1 metre per day, though there was a large source of beetles within 50 metres.

Smith and Whittaker (1980b) have described a link between the feeding behaviour of adult *G. viridula* and the phenology of dock plants. After dock plants flower and the stem leaves begin to die back, any beetles on the plants begin to move up the plant stems and can eventually be seen feeding on the flowers and seeds. When the next set of basal leaves are produced the population can move down the plants and feed on these. These authors also reported that adults avoid laying eggs on old, senescent leaves. When new basal leaves are being produced eggs are laid on these rather than on the old, dying stem leaves that may still be present.

Although *G. viridula* will feed on both *Rumex obtusifolius* and *R. crispus*, when given a choice of leaf discs of the two species, it was found that larvae ate three times as much *R. obtusifolius* as *R. crispus*. When the experiment was repeated using adults instead of larvae, there was some indication that preconditioning could be important. Adults that had previously been reared solely on *R. obtusifolius* consumed more *R. obtusifolius* subsequently than did beetles reared on *R. crispus* and then transferred to *R. obtusifolius*. It was also found that in the field, where *R. obtusifolius* and *R. crispus* occur together, the number of egg clutches laid on approximately 100 plants each of *R. obtusifolius* and *R. crispus* examined on a line transect were in the ratio of 9:1. In laboratory experiments this ratio was 38:13 (Bentley and Whittaker, 1979).

The two species of *Rumex* were found to respond differently to grazing by *Gastrophysa viridula*. Experiments were carried out in which the plants were grown in conditions of intra- or inter-specific competition, with or without grazing by *G. viridula* adults and larvae (Bentley and Whittaker, 1979). Moderate levels of grazing had no significant effect on plants of either species when competing intraspecifically. However, heavy grazing significantly reduced leaf area, leaf dry weight , root dry weight and whole plant dry weight of *R. obtusifolius* and *R. crispus* grown alone. *Rumex obtusifolius* responded to heavy grazing by an increase in the root:shoot ratio, whereas *R. crispus* responded by a decrease in this ratio. In the experiments, *R. obtusifolius* grew better when competing interspecifically with *R. crispus* than when competing intraspecifically, whilst the reverse was found for *R. crispus*. Levels of grazing by *G. viridula*, which had no significant effect on either species when grown alone, resulted in extensive damage to *R. crispus* when the two species were growing interspecifically. Damage to *R. crispus* was higher than expected because *R. crispus* responds by a reduction in the root:shoot ratio, thus making more material available for grazing, whereas *R. obtusifolius* responds by an increase in the root:shoot ratio, thus protecting material from grazing below ground.

Bentley, Whittaker and Malloch (1980) also investigated how the performance of the two species of *Rumex* plants are influenced by grazing by *Gastrophysa viridula*. In two experiments grazing was controlled to simulate the grazing pressure that might be encountered in natural conditions. In one of these experiments, pairs of *R. obtusifolis* and *R. crispus* were planted, while in the other experiment naturally occurring pairs of these plants growing close together were used. In a third experiment, naturally occurring *Rumex obtusifolius* plants were grazed by the naturally occurring *G. viridula* population. Both seed number (where counted) and seed dry weight were significantly reduced in the grazed plants of *R. obtusifolius* as compared with ungrazed controls (only seed dry weight was measured in the experiment with naturally occurring *R. obtusifolius* plants and naturally occurring *G. viridula*). Seed numbers of *R. crispus* were not significantly affected by grazing, but seed dry weight was significantly reduced in the case of naturally occurring plants subjected to controlled grazing. Cideciyan and Malloch (1982) showed that the competitive success of *R. obtusifolius* and *R. crispus* plants was affected by the size of the seeds from which they were grown.

From their observations on the effects of grazing by *G. viridula* on the growth of *R. obtusifolius* and *R. crispus* Bentley and Whittaker (1979) suggested that grazing by this beetle may sometimes affect the distribution of *R. crispus*, especially when the plant is growing in the presence of *R. obtusifolius*. Whittaker (1982) subsequently considered the possibility that the virtual absence of *Rumex crispus* on a shingle bank near Lancaster could be a result of grazing by *Gastrophysa viridula*.

He reported the results of an experiment in which *R. crispus* plants were planted out on the shingle, interspersed with already-existing *Rumex obtusifolius*. Half of the *R. crispus* plants were kept free of *G. viridula* grazing by removing the beetles by hand. Grazing was allowed on the remaining *R. crispus* plants caused by *G. viridula* dispersing from the *R. obtusifolius* plants. Dispersal occurred fairly quickly and resulted in a mean of three egg clutches per plant at the height of oviposition.

The experimental plants of *R. crispus* were harvested at the end of each *G. viridula* generation. It was found that after each of three harvests there was a significant reduction in both shoot and root dry weights of *R. crispus* when grazed by *G. viridula* compared with ungrazed controls. After one harvest, when the population of *G. viridula* had been at a low density, reductions in the weight of grazed plants were not significant.

None of the *R. crispus* plants were killed by grazing. However, during the winter and spring following grazing by *G. viridula*, the experimental *R. crispus* plants were flooded. Survival of previously grazed plants was found to be approximately one-third that of plants previously protected from grazing. The authors attributed this result to the reduction in rooting volume in grazed plants. They attributed the virtual absence of *R. crispus* on the shingle bank to the combined effects of grazing by *G. viridula* and flooding.

Smith and Whittaker (1980a,b) have reported that in some types of habitat *G. viridula* can achieve high population densities resulting in defoliation of the host plant, whereas in others its numbers are always low and it may be unable to complete its full potential three generations. Smith and Whittaker (1980a) found that consistent differences in population numbers could be maintained between habitats separated from one another by distances of only a few metres. They therefore investigated the effects of different types of background vegetation on the population dynamics of *G. viridula*.

Four habitats were studied. One was part of a hay field where the docks were the only dicotyledons present and so were growing effectively as a monoculture. In addition there were three experimental plots which were intended to represent the successional changes occurring in the vegetation colonizing disturbed ground and on which the diversity and maturity of the background vegetation varied. An inverse relationship was found between the diversity and maturity of the background vegetation and the survival of the beetle populations. The major mortality factor appeared to be operating on the second to third instar larvae across all habitats, but the authors reported that habitat type had little effect on egg and first instar larval mortality. However, abundance curve data for *G. viridula* eggs were calculated using numbers of egg clutches rather than individual eggs. They therefore did not give a wholly accurate reflection of egg mortality and any effects of cannibalism of eggs would not have been taken into account.

In a subsequent paper, Smith and Whittaker (1980b) examined the possible mechanisms that may have contributed to the observed effects of habitat type on the population dynamics of *G. viridula*. They found that predator numbers on the plants and, where it could be measured, predator pressure, increased with increasing plant diversity. They concluded that *G. viridula* survives best on communities at an early successional stage due to the influence of predation. They also concluded that populations of *G. viridula* survive well in hay meadows because of periodic mowing or grazing which prevents the dock population from becoming synchronized in seed production and leaf loss.

On the experimental plots, leaf area consumption was never greater than 5% of the standing crop foliage. Abundance curve data are presented for only the diverse vegetation plot. Here the peak mean numbers of animals per plant were 2 adults, 8 egg clutches, 47 first instar larvae, 18 second instar larvae and 13 third instar larvae. On the hay field plot, overall consumption was 45% of the peak standing crop foliage. Smith (1977) had also found that a natural population of *G. viridula* feeding on *Rumex obtusifolius* removed 45% of the peak standing crop of gross leaf area. Unfortunately, the distance curve data given by Smith and Whittaker (1980a) for the population of *G. viridula* on the hay field plot are given as numbers of adults, eggs and larvae per $0.25m^2$. No indication is given of the number of *R. obtusifolius* per unit area, so the density of beetles per plant cannot be ascertained from these data.

G. viridula has been investigated as a possible control agent for broad-leaved docks. As has already been mentioned, *Rumex obtusifolius* is a common species of pastureland. It is however a weed species and therefore of economic importance. The commercial control of dock in Britain is often attempted by using the systemic herbicide asulam (Anon. 1980). The long-term effectiveness of this herbicide is variable however. Speight and Whittaker (1987) have investigated the possibility that the use of *G. viridula*, in conjunction with asulam, could increase the effectiveness or decrease the cost of using asulam on its own.

In laboratory experiments they found that asulam is generally non-toxic to

G. viridula, except in reducing oviposition and rate of development of beetles that have ingested asulam-contaminated tissue during larval growth. However, in field experiments it was observed that asulam-treated plants were not a good environment for *G. viridula* probably because of the lack of high quality foliage rather than a direct effect on the beetles themselves. In the field, the total, shoot and root dry weights of asulam-treated plants were significantly lower than those of untreated plants. Grazing alone did not kill plants, but it significantly reduced the dry weight of asulam-free plants. However, it did not further reduce the dry weight of asulam-treated plants.

The weakening of dock by asulam at the manufacturer's recommended concentration resulted in future generations of *G. viridula* failing to develop. The extent of this depletion in beetle numbers was due in part to the timing of the spraying in relation to the stage of development of the beetle. In the light of these observations, two recommendations were made which could improve the efficiency of a dock control programme. Firstly, spraying with asulam should take place during beetle pupation or adult emergence. Secondly, any reduction in beetle numbers arising from the effects of asulam on the plants could be counteracted by introducing beetles reared indoors that are out of synchrony with wild populations.

Cannibalism

As far as I am aware, there are no published accounts of cannibalism in *Gastrophysa viridula*, and I know of only three other species of chrysomelids in which cannibalism has been reported. Larvae of *Gastrophysa polygoni* cannibalize eggs from within their own clutches (Sotherton et al., 1985), *Labidomera clivicollis* eat conspecific eggs and other larvae (Eickwort, 1973) and in *Plagiodera versicolora* larvae cannibalize other larvae (M.J. Wade and F. Breden, personal communication cited in Pasteels *et al.*, 1986). This does not necessarily give a true indication of the extent of cannibalism in chrysomelid beetles. In fact, cannibalism is known to occur in several species of chrysomelids, but these findings have not been published (J. M. Pasteels, personal communication). The scarcity of published accounts of cannibalism in this group of insects may be a reflection of a more general situation in which cannibalism is either overlooked, or is not considered to be an important aspect of an animal's life history worthy of closer examination.

This view of cannibalism has quite a long history and the thinking of early ethologists may have had a large part to play in its firm establishment. Ethologists took the view that intraspecific killing and predation are rare events in nature and that animal fighting is restrained by ritual and bluff. The statement made by Eibl-Eibesfelt (1961, p.112) that "fights between individuals of the same species almost never end in death and rarely result in serious injury to either combatant" typifies this way of thinking. Likewise, Lorenz (1966, p.38) declared that he "never found that the aim of aggression was the extermination of the fellow members of the species concerned." The early ethologists viewed these observations as being in keeping with their ideas that actions should have evolved for the good of the species. More recently, game theorists have attempted to explain why lethal weapons, killing and cannibalism "are not as common" (Dawkins, 1976, p.72) as might be expected. Maynard-Smith and Price (1973, p.15) stated that "intraspecific conflicts are usually of a 'limited war' type, involving inefficient weapons or ritualized tactics that seldom cause injury to either contestant." Even though game theorists view behaviour from a very different evolutionary perspective, they still seem to have focused on the idea that intra-specific predation is apparently rare.

With such eminent biologists of their time expressing such strong convictions, it is perhaps not so surprising that cannibalism has often been overlooked or considered to be either a laboratory artifact (e.g. Colinvaux, 1973) or aberrant behaviour occurring only in unusual circumstances and in only a few species (e.g. Way, 1966).

However, since the mid-1970s, cannibalism has become increasingly recognized as being a normal and common phenomenon in many natural populations. This has been greatly helped by the publication of two reviews

(Fox, 1975 and Polis, 1981) which brought together much of the literature concerning cannibalism. Polis (1984) also published a review concentrating on intraspecific predation in invertebrates. These reviews left little doubt that cannibalism is extremely common and an important aspect of the biology of many species. Cannibalism has been reported in more than 1,300 species (Jones, 1982) ranging across many different groups of animals : protozoa, planaria, rotifers, snails, copepods, centipedes, spiders, mites, insects, fish, anurans and birds and mammals (see references in Fox, 1975; Polis, 1981, 1984).

Cannibalism commonly takes the form of older animals eating conspecific eggs (as in *G. viridula*) or newborn. In fact, Polis (1984) stated that in invertebrates, apart from species in which sibling cannibalism occurs, adults and older animals are nearly always more cannibalistic than younger animals. He attributed this finding to the fact that in invertebrates predation intensity usually increases with the size of predator because of an increased attack rate and feeding capacity and a decreased handling time. Polis (1981) reported that intraspecific egg-eating was found in more than 80 families, occurring in almost every major group of egg-laying animal : gastropods, spiders, nonsocial insects, social insects, fish, amphibians and birds. Cannibalism of newborn is also widespread. Reviews of infanticide are given by Hrdy (1979) and Sherman (1980), but it should be noted that not all infanticide is cannibalistic.

As Polis (1981, 1984) noted, eggs and newborn animals are relatively defenceless unless guarded by a parent, and at the same time the risk to the cannibal of injury or death from animals in these early developmental stages may be very low. This low risk of injury probably explains why cannibalism most commonly involves larger or stronger animals preying on smaller or more vulnerable conspecifics. However, it is interesting to note that several authors have reported adaptations that reduce cannibalism on eggs and young animals. For example, temporal or spatial separation from adults (Polis, 1980; Tschinkel, 1978), behavioural mechanisms (Lonsdale *et al.*, 1979) and morphological adaptations (Duelli, 1981) have been reported to decrease the probability that eggs and young will be cannibalized (see also references in Polis, 1984, pp.89-90).

Cannibalism of adults by other adults is known but does not occur so commonly (Polis,1981). Perhaps one of the main reasons why cannibalism has been thought in the past to be relatively rare is that both ethologists and game theorists have focused their attention on aggression between adult animals where the costs of retaliation could be very high and where cannibalism is least likely to occur. For example, Dawkins (1976, p.89) comments that "adult carnivores are never to be seen actively pursuing other adults of their own species with a view to eating them." (In fact, this is not quite true. Polis (1981) reported 7 cases of carnivorous mammals in which adults were eaten by other adults of the same species. But again, the majority of cases of cannibalism in this group consisted of adults preying on immature animals and cubs.)

Interactions involving older animals eating apparently defenceless stages such as eggs are not so obviously "aggressive" as the sight of one adult carnivore threatening another- especially since there is no way that an egg can actively fight back. These types of interactions do not have the same attraction of posing the intriguing puzzle of why animals apparently capable of escalating a fight rarely do so. Perhaps for this reason, these less overtly dramatic interactions have not received the same intensity of thought and investigation that has been bestowed on more spectacularly aggressive interactions.

It has often been found that females are more cannibalistic than males within a particular species (see Polis, 1981; 1984). However, it is often difficult in invertebrates to determine the sex of immature animals - such is the case in *G. viridula*, for example. Nevertheless, Polis (1984) found that in 87% of cases in which it had been possible to identify the sex of invertebrate individuals, females were more cannibalistic than males on eggs and young - he did not include cases of female filial cannibalism.

Cannibalism has been studied from several different points of view. In many cases the effects of cannibalism at the population level has been the subject of investigation. There are often many dificulties involved when trying to evaluate the population consequences of cannibalism. For instance, the importance of cannibalism cannot always be judged simply by determining the extent to which conspecific individuals make up the diet of animals in a particular population (Fox, 1975). Pritchard (1964) and Lawton (1970) found that cannibalism among dragonfly and damselfly nymphs respectively was relatively uncommon and therefore concluded that it was unimportant. And yet in the freshwater leech, *Erpobdella octoculata*, where conspecific eggs constituted only about 0.2% of the annual diet, cannibalism was considered to be the main population regulatory mechanism (Eliot, 1973). Chevalier (1973) reported that the 88% mortality among young walleye could be explained by less than 3% cannibalism in the diet of the adult fish.

In situations in which food is in short supply, it is possible that both cannibalism and starvation could produce similar patterns of survival. In such cases it would be necessary to have independent measurements of cannibalism to determine which of theses two alternatives was responsible for the observed survivorship patterns (Fox, 1975). However, it is not always possible to obtain accurate estimates of the extent of cannibalism in the field. For example, Sotherton *et al.* (1985) resorted to estimating the rate of egg cannibalism by larvae in clutches of *Gastrophysa polygoni* eggs from observations in the laboratory. This was because in the field they were unable to distinguish between infertility and cannibalism of viable eggs as causes of mortality during the egg stage. In addition, these investigators were not able to take into account the possibility that some eggs within clutches may have been eaten by larvae from other clutches since this form of cannibalism could not be distinguished from predation of eggs by other species.

Cannibalism may also be difficult to detect if it takes place only during certain restricted periods of time. For example, Brower (1961) found that most larvae of the butterflies *Danaus plexippus* and *D. gilippus* were cannibalistic only during the first few hours after hatching.

Nevertheless, despite the many difficulties, cannibalism has been found to be a major cause of mortality in many species ranging from copepods and dragonflies to fish, birds and carnivorous mammals (see Polis, 1981, 1984 and references therein). In some cases cannibalism by older individuals can cause almost complete elimination of eggs and/or young produced by a population. This may result in dramatic fluctuations in recruitment and skewed age or size distributions. This happens commonly in fish (e.g. Ricker, 1954; Radovich, 1962; Forney, 1976; Holcik, 1977; Popova and Sytina, 1977; De Angelis *et al.* 1979). It also occurs in several families of insects and in some species of polychaete and molluscan filter and deposit feeders (Fitch, 1964; Woodin, 1976).

Polis (1981, 1984) reported that cannibalism is often a function of density and that in more than 40 examples, intraspecific predation was found to act as a density dependent regulator of population size. Polis (1981, 1984) also reported that cannibalism may result in more or less regular spacing among territorial individuals or individuals defending a discrete food resource. For example, cannibalism is reported to result in overdispersion in the larvae of some insect orders (e.g. Crombie, 1944; Corbet and Griffiths, 1963; Nikolskii, 1969; Andrewartha, 1971; Jonasson, 1971; Gould *et al.* 1980) and in many benthic invertebrates (Johnson, 1959; Jonasson, 1971; Woodin, 1974). Owls (Eckert, 1974) and hyaenas (Kruuk, 1972) will also kill conspecifics in defence of territories. Regular distribution of parasitoid Hymenoptera can result from larvae killing other conspecific larvae to gain sole access to an insect host's body (Salt, 1961; Fisher, 1970; Choudhuri and Bagh, 1974). Similarly, regular distribution of granivorous insects (Crombie, 1944; Coyne, 1968) including seed-feeding bruchids (Wang and Kok, 1986) can result from cannibalism to eliminate other conspecifics from individual seeds.

In some populations, cannibalism may increase in times of prey shortage and decrease when there is an abundance of prey, thus altering the population size in response to a varying food supply (Fox, 1975; Polis, 1981, 1984 and references therein; Pajunen, 1983). Cannibalism may thus enable populations of desert scorpions (Polis, 1980), sheep blowfly (Ullyette, 1950) and other animals (Banks, 1956; Kaddou, 1960; Beaver, Croft and McMurty, 1972; 1974; Duelli, 1975) to persist when food is short in a fluctuating environment.

It has been suggested that cannibalism also increases stability in predator-prey systems. Sempala (1983) studied interactions between *Aedes africanus* and two mosquito predators, *Toxorhynchites brevipalpis conradti* and *T. kaimosa.* Cannibalism appeared to play an important role in the regulation of the population sizes of the two species of *Toxorhynchites*, thus limiting the build-up of these predator populations to a level at which they could significantly deplete the prey population. White and Huffaker (1969) studied interactions between a lepidopteran species *Anagasta kuhniella* and the predatory mite *Blattisocius tarsalis*, both of which are cannibalistic. They suggested that cannibalism increases stability in this predator-prey system by damping the magnitude of population fluctuations.

Despite the fact that in their reviews Fox (1975) and Polis (1981,1984) concentrate on examples where cannibalism is considered to be an important factor in regulating the numbers of individuals in populations or in maintaining population stability, intraspecific predation may not always be important in these respects. For example, although egg cannibalism occurs in the thistle-feeding lady beetle *Henosepilachna pustulosa*, Nakamura and Ohgushi (1981) reported that the results of key-factor analysis indicated that the stabilization of population size was attained through density-dependent regulatory processes operating in inter-patch dispersal and in oviposition by overwintered adults. Egg mortality was not important. Sotherton *et al.* (1985) studied the numbers of a chrysomelid beetle, *Gastrophysa polygoni*, found in cereal fields in southern England over a period of three years. They found that although no potentially regulating factors could be identified, the key factor operated on the egg stage. However, they reported that predation, in particular by Carabidae and Staphylinidae, was by far the most important egg mortality factor. They considered that cannibalism was not important.

In *Gastrophysa viridula* Smith and Whittaker (1980a) reported that the key factor operated on the second- to third-instar larvae and mortality during the egg stage was the least important factor. However, abundance curve data for *G. viridula* eggs were calculated using numbers of egg clutches rather than individual eggs. Any effects of cannibalism could not therefore be taken into account.

Mathematical models have been used to investigate the effects of cannibalism on population dynamics. Bobisud (1976) published an analysis of a predator-prey system in which the prey species has a three-stage life cycle and is capable of adopting either egg cannibalism by larvae or larval cannibalism by adults. The stated aim of this paper was to investigate the possibility of cannibalism evolving in the prey population. However, Stenseth and Reed (1978) and Reed and Stenseth (1984) have criticized Bobisud's paper on the grounds that his results do not give an argument relevant to the evolution of cannibalism unless the argument of group selection is invoked.

Nevertheless, Bobisud's paper does tackle the problem of whether the equilibrium densities will increase or not, should cannibalism prove to be favoured by natural selection. He concluded that sufficiently restricted cannibalism can result in an increase in the number of adult prey present at the stable equilibrium point when cannibalism decreases the vulnerability of a stage subject to predation or increases overall productivity.

Several papers have been published which investigate predator-prey interactions in which the prey species is age-structured and the predators consume only the eggs of the prey. The first of these is by Gurtin and Levine (1979) and investigates a model analogous to the Lotka-Volterra equations. The authors reported growing amplitude oscillations which lead to the extinction of the prey species and subsequently of the predator species since no alternative food source is included in the model.

Subsequent models developed by Levine (1981), Thompson *et al.* (1982), Frauenthal (1983) and Coleman and Frauenthal (1983) introduced more realistic effects such as a prey carrying capacity, mutual interference between the predators and predator appetite satiation. Introducing these factors leads to a stable equilibrium with both species present in these models. Diekmann *et al.* (1986) have developed a model of density-dependent egg cannibalism in an age-structured population from which they deduce that cannibalism may promote oscillations.

Mathematical models have been used specifically to investigate the effects of cannibalism in the population dynamics of flour beetles of the genus *Tribolium*. *Tribolium* species (Tenebrionidae) were first used in ecological research by Chapman (1928). Whilst the total number of beetles was observed to reach a steady state density when grown in a continuous culture, the numbers of eggs and larvae showed regular cycles which Chapman (1933) considered to be similar to those predicted for predator-prey systems by Volterra (1931).

Although there has been some controversy over the relative importance of such factors as cannibalism, crowding, fecundity and conditioning of the medium (e.g. Chapman, 1928; Boyce, 1946), it is now widely accepted that cannibalism is a major factor in regulating laboratory populations of *Tribolium* (e. g. Mertz and Robertson, 1970). Cannibalism can take the form of adults and larvae eating eggs, larvae eating other larvae and adults eating pupae and callows (Daly and Ryan, 1983).

Following the original work of Chapman (1928), an enormous amount of experimental data has been gathered with regard to the population cycles of

Tribolium, notably by Thomas Park and his students at the University of Chicago (see Park *et al.*, 1964). Such a wealth of experimental studies has in turn stimulated many theoretical studies of the population dynamics of *Tribolium* (Sokoloff, 1974; pp. 536-562). Recent papers have attempted to provide a unifying theoretical framework to study the effects of various factors on the population dynamics of these animals.

Hastings (1987) and Hastings and Costantino (1987) have developed a model consisting of a system of nonlinear Volterra integral equations which can be studied using Hopf bifurcation techniques. Since the lifetime of the adults is several times longer than the duration of the life cycle, they assume that interactions between eggs and larvae occur on a faster time scale than those involving changes in adult numbers. Their model focuses on the egg-larval interaction that includes cannibalism and incorporates basic biological features of *Tribolium* biology. They further assume that the magnitude of larval cannibalism is not dependent on the age of the larvae.

With this model they were able to determine the effect of varying cannibalism rates as well as fecundity, survival and lengths of egg and larval stages at stability. They demonstrate that their model possesses both stable oscillations and a stable equilibrium over a significant range of parameters. Hastings (1987) proved the existence of multiple attractors in this model. However, it remains to be seen if this result would hold true for the more realistic case in which the rate of cannibalism is dependent upon the age of the larvae (Desharnais and Liu, 1987).

Desharnais and Liu (1987) have investigated the population dynamics of *Tribolium* using a general model developed by Liu and Cohen (1987) which allows age-dependent interactions to suppress the reproduction and survivorship of each age-class. This model is based on the standard matrix approach and includes egg cannibalism by larvae and adults and pupal cannibalism by adults. They also include an assumption that egg cannibalism rates by larvae increase linearly from zero as larvae get older. They applied the model to the dynamics of laboratory populations of *Tribolium castaneum*, using data from the literature to estimate the parameters of the model.

They found that the model simulations agreed fairly well with the experimental data of Desharnais and Costantino (1980) where the demographic stability of laboratory populations of the corn oil sensitive strain of *T. castaneum* was assessed by perturbing populations which were near equilibrium. Many of their results were consistent with the findings of Hastings (1987) and Hastings and Costantino (1987). They found that increases in the rates of cannibalism by larvae tended to be destabilizing whereas increases in the rates of cannibalism by adults were stabilizing. In both models the period of the oscillations was found to be approximately equal to the developmental period. They also found that the oscillations of adult numbers were small, but that the numbers of eggs and larvae showed large oscillations.

Although mathematical models necessarily compromise between analytical tractability and the full description of a realistic situation, they have enabled some insight to be gained into the effects of cannibalism on population dynamics.

The framework within which cannibalism is investigated throughout this thesis is the possible importance of individual fitness benefits and kin selection in the evolution of cannibalistic behaviour. If cannibalism has a genetic basis, it will have evolved and be maintained through the process of natural selection (Polis, 1981, 1984). The possible factors that may have selected for the evolution of cannibalism have received increasing attention in recent years (e.g. Eickwort, 1973; O'Connor, 1978; Stenseth and Reed, 1978; Jones, 1982; Polis, 1981, 1984; Skurdal *et al.*, 1985; Stenseth, 1985; Baur and Baur, 1986 and Baur, 1987).

Several types of evidence have been obtained which indicate that there may be a genetic basis for cannibalism in many species. For example, breeding strains, races or populations with different propensities for cannibalism have been found in several species (Fox, 1975; Polis, 1981,1984 and Baur and Baur, 1986). Fox (1975) considered that the restriction of cannibalism to a particular life-history stage or sex within any one species and differences in the cannibalistic tendencies of closely related species also provide evidence for a genetic basis for cannibalism.

From the point of view of individual selection, the important thing to consider is whether or not cannibals may gain some personal benefit that results in a higher reproductive success than that of non-cannibals - where reproductive success is defined as an animal's lifetime production of offspring that themselves survive to reproductive age. Possible benefits to cannibals include direct nutritional advantages (in terms of energy or some specific nutritional element such as protein) resulting in increased developmental rates, growth, survival and reproduction, the elimination of potential competitors and possibly the elimination of potential conspecific predators (Fox, 1975, Polis, 1984).

Polis (1984) pointed out that the elimination of potential conspecific predators may be particularly important in some invertebrates. Arthropods periodically pass through immobile stages when they are especially vulnerable to predation by conspecifics. By killing conspecifics, individuals may reduce the probability that they themselves will become the victims of cannibalism during quiescent stages. Similarly, mothers may reduce the risk of their offspring becoming the victims of cannibalism by eating conspecifics in the area surrounding their young. For example, scorpion mothers eat conspecifics in the territory around their own burrows thus enabling their offspring to disperse into an area that is more or less free of older conspecific predators (Polis, 1984). Cannibalism would be expected to have evolved if it results in cannibalistic individuals having a greater fitness than non-cannibals.

However, if cannibalism involves killing and eating relatives, a more complicated situation has to be considered. Sibling cannibalism and filial cannibalism are both fairly common (Polis, 1981,1984). In these cases, it is not sufficient to consider only the individual fitness benefits gained by cannibals as compared to non-cannibals. In both sibling cannibalism involving full-siblings and filial cannibalism, the victim has a 50 % probability of sharing a given rare gene in common with the cannibal. For any cannibalistic interaction involving relatives, the more distantly related the cannibal and victim are to each other, the lower the probability of their sharing a rare allele in common. For example, individuals cannibalizing their cousins will have only a 25% probability of sharing a rare allele with their victims.

Hamilton (1964a,b) used the idea of "inclusive fitness" to define the conditions under which a gene might spread through a population, taking into account the effect that individuals carrying the gene might have on individuals of different degrees of relatedness. Inclusive fitness refers not to the reproductive success of a single individual, but to the success or otherwise of a whole genotype compared to some alternative genotype. The fitness of a genotype is increased relative to that of another if the number of copies of that genotype passing to the next generation is greater than the number of copies of the alternative genotype.

If cannibalism has a genetic basis, the average fitness of a cannibalistic genotype will be greater than that of a non-cannibalistic genotype, providing that the cannibals produce more than enough offspring to compensate for the loss of the causative gene or genes in their victims. As the genetic relatedness between cannibal and victim becomes more distant, a smaller advantage to the cannibal is required before the cannibalistic genotype will be selected for. When thinking about the possible factors that may have selected for cannibalism involving relatives it is therefore important to consider not only individual fitness benefits to the cannibals themselves, but also the genetic cost of killing a relative with a particular probability of sharing the causative gene or genes.

Parental manipulation has also been considered as an important factor in the evolution of cannibalism. Alexander (1974, p.337) stated that "parental manipulation of progeny refers to parents adjusting or manipulating their parental investment, particularly by reducing the fitness of certain progeny in the interests of increasing their own inclusive fitness via other offspring". Parents eating their own offspring is a normal occurrence in some species.

For example, parental manipulation occurs in many social insects where the parent sacrifices some offspring to increase the fitness of itself and other offspring. Founding queens often eat their own eggs or feed them to the first group of larvae and the brood may be used as a food reserve for times when other food is in short supply to keep the queen and workers alive (Wilson, 1971, Polis, 1984).

For the case of sibling cannibalism, where individuals eat eggs, embryos or newborn animals from within their own clutch, it has been suggested that the victims function as a temporary food store for their cannibalistic relatives. Alexander (1974) suggested that the use of smaller offspring as food supplies for older offspring may have evolved under conditions where there is no easy way to convert maternal tissue directly into food for offspring as, for example, female mammals do. Intrabrood or intraclutch cannibalism may function to increase parental reproduction by resulting in well-nourished offspring in cases where females are unable to supply enough nutrients in their eggs or to otherwise feed young as they develop. This type of cannibalism has been discussed by R. D. Alexander and he has created a special term for the phenomenon - the "ice box hypothesis". Polis (1984, p.100) summarizes Alexander's idea in the following manner:- "Such intrabrood cannibalism is characterized by the use of some offspring as extended parental investment sacrificed to profit other offspring : Victims essentially function as packages of live meat for their kin".

Polis (1984) noted that the production of infertile "trophic" eggs represents an extreme form of the food cache strategy. He found examples of over 100 species of invertebrates in which siblings eat non-viable eggs from within their own broods or clutches. Alexander (1974) suggested that brood reductions in birds associated with asynchronous hatching, which may or may not involve cannibalism, probably function to increase parental reproduction.

Although these ideas provided the motivation behind the research carried out for this thesis, it is important to realize that not all cannibalism is necessarily adaptive and a product of natural selection. Some cannibalism may be maladaptive resulting from stress or accidents, or it may occur as a by-product of the normal feeding habits of some species such as filter-feeding corals, copepods and several species of bivalve molluscs and suspension and deposit feeders (Polis, 1981, 1984; Meffe and Crump, 1987). These possibilities were also kept in mind when interpreting the results of experiments and observations.

General plan of the thesis

A more detailed introduction to the various aspects of cannibalism in *G. viridula* covered by the thesis will be given at the beginning of each chapter and section. A brief outline of the material contained in each chapter is given here.

Chapter 2 deals with aspects of within-clutch cannibalism. In the first section the extent of within-clutch cannibalism is examined with a view to gaining some insight into the importance this behaviour might have in the life-history of *Gastrophysa viridula*. In the second section of this chapter an experiment is presented which was designed to determine whether potentially viable or non-viable eggs are eaten within clutches by other clutch members. In some animals infertile "trophic" eggs are laid which serve as food for the hatchlings from fertile eggs. This has been reported in marine snails, spiders and several orders of insects (Polis, 1981, 1984). In other species, animals are known to feed on dead conspecifics. This happens, for example, in many species of anurans (Crump, 1983). Although eating non-viable or dead conspecifics may be important from the point of view of the potential fitness benefits gained by individuals, the consequences of eating potentially viable members of an animal's own species will have very different consequences in terms of the inclusive fitness of the animals. The inter-relationships between clutch size, the degree of asynchrony of hatching and the extent of within-clutch cannibalism are examined in the third section of Chapter2.

Sperm competition, the situation in which the sperm of more than one male compete for the fertilization of a female's ova (Parker, 1970b) in *G. viridula* is the subject of **Chapter 3**. This was investigated with a view to ascertaining the probable relatedness of cannibals to their victims in cases of within-clutch cannibalism.

In **Chapter 4** the relationship between the age of *G. viridula* eggs and their susceptibility to cannibalism by first instar larvae is examined. The significance of this relationship is discussed in terms of the protection of eggs both against within-clutch cannibalism and against cannibalism by larvae from other clutches (inter-clutch cannibalism). The findings raised the interesting possibility that chemical defences, normally thought of as having evolved as a means of protection against predation from other species, may also be involved in protecting *G. viridula* eggs against predation from within their own species. A discussion of these ideas and a preliminary investigation are presented in the second section of Chapter 4.

In **Chapter 5** the personal nutritional benefits gained by cannibals is explored. It has been noted that many instances of cannibalism are found in species that are normally herbivorous, including butterflies, chrysomelid beetles and bark beetles. And although cannibalism has often been found to be a response to food shortage or a high population density, some herbivorous insects are known to maintain their cannibalistic habits at low population densities even when their plant food is in abundant supply (Fox,1975). This suggests that conspecifics may in some way be nutritionally superior to the usual plant food of some herbivores. For example, eating conspecifics may provide a particular nutritional component, such as protein, which is either lacking or present in very small amounts in an animal's diet of plant material. The relative values of diets consisting of conspecific eggs, dock leaves or combinations of both for the development, growth, survival and fecundity of *G. viridula* larvae are considered in this chapter. As a by-product of these investigations, information was gained regarding the relationship between lifetime egg production and female size. An interesting pattern in the numbers of eggs laid by females in successive clutches was also discovered and these findings are also presented in Chapter 5.

Aspects of inter-clutch cannibalism are considered in **Chapter 6**. The first section deals with the possible influence over the extent of inter-clutch cannibalism that females may exert through the position in which they lay their eggs in relation to previously laid ones. *G. viridula* larvae in their second and third instars move quite widely across several leaves within a dock plant. If they remain cannibalistic throughout all three larval instars, the potential for inter-clutch cannibalism would obviously be much greater than if they were cannibalistic only during their first instar when they generally stay on the leaf on which they hatched. The duration of the cannibalistic tendencies in *G. viridula* larvae is the subject of the second section in this chapter. Since the relatedness between interacting individuals is crucial to the idea of inclusive fitness, an experiment was carried out to determine if *G. viridula* larvae distinguish between relatives and non-relatives as their victims. This experiment is presented in the third section of Chapter 6 and the implications of the findings are discussed.

Detailed discussions of each topic are given in the appropriate chapter and section. The final chapter entitled "General Discussion" is included mainly to bring together in the one place the major findings, conclusions and speculations arising from the work of the thesis. Work carried out as part of this research project, but not included in the main body of the thesis is also briefly described in the General Discussion. Finally, a section is included which aims to point out areas which may be worthy of closer investigation from the viewpoint of determining the importance of various factors in the evolution of clutch size in *Gastrophysa viridula.*

Animal stocks, laboratory conditions and statistics

Two stocks of *G. viridula* beetles were set up in the laboratory. One stock, referred to as the "Field" stock throughout the thesis, was derived from beetles collected from a field station at Lancaster University (O.S. grid reference SD 489 569). The other stock, equally unimaginatively referred to as the "Roadside" stock, was derived from beetles collected from a wide roadside verge, five miles from the "Field" site (O.S. grid reference SD 531 637).

The beetles were kept in perspex fish aquaria or large pyrex dishes with perspex lids. The containers were lined with damp, sterilized sand and broad-leaved dock (*R. obtusifolius*) leaves were provided as food. Plants were grown from seed in a greenhouse for this purpose and, except in summer when they grew under the natural light / dark cycle, the plants were provided with a 16h:8h light:dark photoperiod. The beetles themselves were maintained under controlled conditions of $20^{\circ} \pm 1^{\circ}$ C. and a 16h:8h light:dark photoperiod. These conditions normally prevent the beetles from entering diapause, and at Lancaster University, where the beetles have been the subject of an extensive research programme over many years, little difficulty has been experienced in maintaining laboratory stocks under these conditions throughout the winter (Dr. J.B. Whittaker, personal communication). However, on several occasions the entire laboratory stocks that I was working with died out. This usually happened in the autumn, but I was unable to discover the cause of the deaths. On these occasions replacement beetles were obtained either from the "Field" site itself or from the laboratory stocks at Lancaster University which were derived from beetles from the same site. (The "Roadside" beetles were not replaced as they were no longer needed for experiments).

Except where otherwise stated, the experiments described in this thesis were all carried out under controlled laboratory conditions of $20^{\circ} \pm 1^{\circ}$ C. and a 16h:8h photoperiod. The food plant used in all the experiments will simply be referred to as "dock leaves". The dock leaves were in fact always from the broad-leaved dock, *Rumex obtusifolius.*

Statistical procedures were carried out using Minitab and SPSSX statistical packages (Minitab Reference Manual (Minitab Release 5.1), 1986; SPSSX User's Guide. Edition 2, 1986). Means are always presented as the mean ± one standard error.

CHAPTER 2

WITHIN - CLUTCH CANNIBALISM IN *G. VIRIDULA*

Outline of Chapter 2

In this chapter, various aspects of within-clutch cannibalism in *Gastrophysa viridula* are considered. In the first section the extent of within-clutch cannibalism is examined. The second section deals with the question of whether viable or non-viable eggs are eaten from within clutches by other clutch members. The relationships between clutch size, asynchrony of hatching and the extent of within-clutch cannibalism are discussed in section three.

2.1. THE EXTENT OF WITHIN - CLUTCH CANNIBALISM AND THE CANNIBALISTIC POTENTIAL OF NEWLY HATCHED LARVAE

INTRODUCTION

As a starting point in the study of egg cannibalism by larvae of *Gastrophysa viridula* it was necessary to know how important this factor is in causing the loss of eggs from clutches. Cannibalism could have quite considerable consequences for the inclusive fitness of mother beetles, cannibalistic larvae and of course, the victims themselves. The magnitude of this effect will depend on the individual benefits gained from cannibalism, the relatedness of cannibals to their victims and the extent to which cannibalism actually occurs in this species. Intra-clutch cannibalism, in which larvae eat eggs from within their own clutch, was looked at first of all.

Egg cannibalism in insects has been most studied in the Coccinellidae and losses of eggs through intra-clutch cannibalism in this group of beetles can be quite high. Banks (1956) reported that in *Coccinella septempunctata* (L.) where the mean number of eggs per clutch was 31.8, 21.4% of eggs were cannibalised by newly-hatched larvae from within their own clutch. Similarly, Pienkowski (1965) found that first-instar larvae of *Coleomegilla maculata lengi* Timberlake ate 21.1% of the eggs within their own clutches having a mean clutch size of 11.8 eggs. In *Cheilomenes lunata* (F.), this type of cannibalism accounted for the loss of 42% of eggs within clutches where the mean clutch size was 16.2 eggs (Brown, 1972). On the other hand, Banks (1956) found that the larvae of *Propylea quatuordecimpunctata* (L.) were responsible for the loss of only 10.6% of eggs from within clutches with a mean clutch size of 6 eggs. Sotherton *et al.* (1985) working on the chrysomelid beetle, *Gastrophysa polygoni* (L.), found that 7.1% of the eggs of clutches in this species were lost through intra-clutch cannibalism.

The extent of within-clutch cannibalism in *Gastrophysa viridula* was studied during an experiment on sperm competition (see Chapter 3). The extent of cannibalism in "normal" clutches was examined in the eggs laid by females mated with two normal males. At the same time, an opportunity arose to compare this with the cannibalism which takes place in similar sized clutches, but where the number of larvae hatching out was abnormally reduced. In addition to the females that had been mated with normal males, others were mated with either two irradiated males or one normal and one irradiated male. Most, but not all, eggs fertilized by irradiated sperm failed to hatch and a range of numbers of larvae hatching out in different clutches was obtained. The clutches where very few larvae hatched out provided an ideal situation in which to test the full cannibalistic potential of newly-hatched larvae. Any discrepancy between the potential for cannibalism found in these clutches and the cannibalism that is actually attained in normally-hatching clutches would require further investigation.

METHODS

The details of the procedures to obtain females mated with normal males, two irradiated males or one of each are given in Chapter 3. After mating, these females were isolated in 9 cm. diameter petri dishes lined with moistened filter paper and supplied with fresh dock leaf. The number of eggs laid in each clutch was counted. The clutch was then transferred, on the piece of dock leaf on which it had been laid, to a 5 cm. petri dish. The filter paper in these petri dishes was kept moist so that the pieces of dock leaf did not noticeably wither or wilt before the larvae had hatched out.

The eggs are susceptible to fungal infections and so all counting and manipulating of eggs was carried out under sterile conditions of a laminar flow cabinet to minimize this risk. Distilled and sterilized water was used to keep the petri dishes moist. Fungal infections are not usually a problem in normally hatching clutches. However, during a trial experiment to determine the most appropriate level of X-ray radiation for the males in the sperm competition experiment, it was noticed that fungal infections often set in after any of the eggs in a clutch had been punctured by larvae and the majority of the eggs remained unhatched. Although fungal infections did not usually set in until at least a day after hatching, it seemed worthwhile trying to minimize the risks of infection since any infection might have affected the numbers of eggs that larvae could eat from clutches, making the estimation of the full cannibalistic potential of larvae unreliable.

"Normal" clutches

Twenty-four hours after hatching, the clutches were examined under a binocular microscope. In the clutches laid by females mated with normal males, the number of larvae that had hatched out was counted and any remaining eggs were examined for signs of attack by the larvae. The mean percentage of eggs attacked per clutch was calculated using arcsine transformed data. The result was reconverted to an actual percentage for presentation. One hundred clutches from 10 females were examined in this way.

Clutches with artificially reduced numbers of larvae hatching out

The numbers of larvae hatching out from clutches laid by females mated with either one or two irradiated males were also counted. In addition, a score of the eggs eaten by these larvae after 24 hours was obtained. Eggs that had not been eaten at all were scored as 0; eggs that had been up to a half eaten were given a score of 0.25 and eggs that had been more than half eaten were scored as 0.75.

Since the point of these observations was to find out the cannibalistic capacities of newly-hatched larvae when they are not limited by the number of eggs available to be eaten, the only clutches considered in the analysis were those in which an arbitrarily chosen amount of eggs remained 24 hours after hatching. Clutches were included in the analysis if eggs amounting to a score of at least 7.5 remained. Since the mean clutch size of these clutches was 42.1 ± 0.4 (n = 36), this represented, on average, roughly one sixth of the eggs in each clutch and was considered to be sufficient to avoid the possibility of larvae being limited in their cannibalism by the amount of eggs available for them to eat. Thirty-six clutches from 7 females mated with two irradiated males and 8 females mated with one irradiated male satisfied this criterion.

The score of eggs eaten per larva in each clutch was calculated. Multiple regression analysis was used to check that the score of eggs eaten per larva was not affected by the number of larvae that hatched out in each clutch or the number of eggs available for eating, calculated as the clutch size minus the number of hatched larvae.

RESULTS

"Normal" clutches

All unhatched eggs in clutches laid by females mated with normal males had been attacked by larvae. Most had had their contents completely sucked out and the egg shells had also been eaten in some cases. A few eggs had been only partially eaten. The mean percentage of eggs attacked per clutch was 4.1 $\pm^{0.6}_{0.5}$ (n = 100). The clutch sizes ranged from 6 to 51 eggs, with a mean of 41.1 ± 0.6.

Clutches with artificially reduced numbers of larvae hatching out

In the clutches laid by females mated with at least one irradiated male and in which eggs amounting to a score of at least 7.5 remained 24 hours after hatching, the mean score of eggs eaten per larva was 1.65 ± 0.17 (n = 36). The clutch sizes ranged from 38 to 46 eggs with a mean of 42.1 ± 0.4. The number of larvae which hatched out in these clutches ranged from 1 to 13 with a mean of 2.6 ± 0.4. Multiple regression analysis showed that there was no significant relationship between the score of eggs eaten per larva and the number of larvae in each clutch or the number of eggs available for eating once the larvae had hatched (Score of eggs eaten = - 0.03 no. of larvae + 0.08 no. of eggs available for eating - 1.375. F=1.86, p=0.17, r.sq.=10.11%, d.f.=2,33). Each first-instar larva, when not limited by the supply of eggs, was therefore able to eat, on average, eggs amounting to a score of 1.65 within the first 24 hours after hatching.

Comparison of the amounts of eggs eaten in "normal" clutches and in clutches with artificially reduced numbers of larvae hatching out

The score of eggs eaten was not obtained from the normally hatching clutches. However, the amounts of eggs eaten in clutches of the two different groups can be roughly compared by allocating each egg cannibalized in the normally hatching clutches a maximum score of 0.75. This seems to be a valid thing to do since most unhatched eggs in the normally hatching clutches were completely eaten. Such a comparison would only be valid if the clutch sizes did not differ significantly between the two groups. The means of the clutch sizes in both groups were compared using a two-sample t-test and no significant difference was found (mean clutch size in normally hatching clutches = 41.1 ± 0.6, mean clutch size in clutches with reduced hatching = 42.1 ± 0.4. t=1.45, p=0.15, d.f.=131).

To obtain the score of eggs eaten per larva, the total score of eggs eaten per clutch was divided by the number of larvae in the clutch. The mean score of eggs eaten per larva in the normally hatching clutches was 0.049 ± 0.006 (n = 100) which was significantly different, using a two-sample t-test, from the mean of 1.65 ± 0.17 (n = 36) obtained from the clutches in which few larvae hatched out ($t=9.65$, $p<0.0001$, d.f.=35).

It is unlikely, however, that all the larvae in a clutch will eat a very tiny portion of an egg each. Although several larvae have been observed feeding on unhatched eggs within clutches, it would have been very difficult to obtain data on the exact numbers of larvae that cannibalized eggs and those that did not. When larvae emerge from their egg shells, they crawl over each other and any remaining unhatched eggs. It is very difficult then to follow individual larvae, and looking at them from above, through a microscope, it is even difficult to tell sometimes whether they are feeding on an egg or not.

However, if the very conservative assumption is made that only one larva in each clutch cannibalizes unhatched eggs, then the means of the total scores of eggs eaten in each clutch can be compared, again using a two-sample t-test. The mean total score of eggs eaten in normally hatching clutches was 1.8 ± 0.18 (n = 100) whereas the mean for clutches in which few larvae hatched was 3.74 ± 0.5 (n = 36). Even by making this unrealistically conservative assumption about the number of larvae which fed on the unhatched eggs, a significant difference is found between the scores of eggs eaten in the two groups (t=3.67, p=0.0006, d.f.=44).

DISCUSSION

Within-clutch cannibalism in *Gastrophysa viridula* is not very extensive. It is more in line with the findings of Sotherton *et al.* (1985) for cannibalism in *G. Polygoni* than with the findings for most coccinellid species (e.g. Banks, 1956; Pienkowski, 1965; Brown, 1972). Sotherton *et al.* stated that cannibalism by first-instar larvae within clutches was not important in *G. polygoni.* The same conclusion might have been drawn for the green dock leaf beetle, except that the potential for cannibalism within clutches has been shown to be greater than what is actually achieved in normally hatching clutches.

The interest of within-clutch cannibalism in *Gastrophysa viridula* lies not so much in its extent therefore, as in the degree to which it is restricted. An explanation for this low level of cannibalism might be provided if larvae were found to eat only non-viable eggs and these eggs were limited in number in normal clutches. This possibility will be explored in the next section.

2.2. THE TYPES OF EGGS EATEN - VIABLE OR NON-VIABLE ?

INTRODUCTION

As seen in the previous section, very few eggs are normally eaten from within clutches by newly-hatched larvae. Nevertheless, it is important to determine whether these eggs are non-viable or if, in the absence of cannibalism, they could have hatched out. If only non-viable eggs are eaten and these are relatively scarce in normal clutches, the limited extent of intra-clutch cannibalism would be easily explained.

It is important to know whether viable or non-viable eggs are eaten from the point of view of the inclusive fitness of both mother beetles and their cannibalistic offspring. If only non-viable eggs are eaten, the fitness considerations that would have to be taken into account would be the nutritional value of eating these eggs in terms of the future survivorship and reproductive potential of the cannibalistic larvae as compared with non-cannibalistic larvae. There is no genetic cost in eating an egg that would not have hatched out anyway. On the other hand, if some viable eggs are also eaten, any nutritional benefits gained from eating these eggs would have to have outweighed the costs of killing a relative for this behaviour to have been selected, or at least for it not to have been strongly selected against (See Chapter 5).

The importance of distinguishing between viable and non-viable eggs as the victims of cannibalism has been realized by several authors. Mrowka (1987) has reported the results of experiments and observations on *Pseudocrenilabrus multicolor*, a maternal mouthbrooding cichlid fish. Spawned eggs are carried in the female's mouth for a period of 10-11 days during which time she does not feed. As a consequence of strong competition during spawning at high density, many female fish have a mixture of fertilized and unfertilized eggs in their mouths. The unfertilized eggs are selectively swallowed and in addition, roughly 15% of the fertilized eggs are swallowed.

The eating of non-viable eggs can be seen as an efficient use of a food resource, but eating fertilized eggs does not at first sight seem to have such a straightforward explanation. Mrowka proposed two reasons why these eggs might be eaten. Firstly, he suggested that these fertilized eggs may have died due to developmental disturbances and so would be treated in the same way as unfertilized eggs. If this were the case, all swallowed eggs would, in effect, be non-viable. Alternatively, he suggested that since hunger exceeding a certain level in the female may cause the whole brood to be eaten (Mrowka, 1984, 1986), females may eat some of their viable offspring in order to save the greater part of a brood. The costs of eating a small proportion of their own offspring may then be outweighed by the benefits of being able to continue to rear the majority of the brood. Another possibility which seems worth considering, but which was not discussed by Mrowka, is that the female fish are simply unable to be completely efficient in selectively swallowing only infertile eggs. Swallowing some fertile eggs along with the infertile ones may be unavoidable and, if such were the case, it would best be regarded as a "mistake" rather than any sort of an optimal strategy.

Fertilized eggs are also eaten in another female mouth-brooder, *Oreochromis leucosticus* (Welcomme, 1967) and similar explanations to Mrowka's for filial cannibalism have been proposed by Liebman (1933) for mouthbrooding cichlids and by Gunter (1947) for a mouthbrooding marine catfish.

Baur and Baur (1986) have observed newly-hatched land snails of the species *Arianta arbustorum* eating eggs from within their own clutches. These eggs included ones in which the embryos were almost fully developed. This finding led the authors to seek an inclusive fitness based explanation for the cannibalistic behaviour. However, although eggs with fully developed embryos were eaten, this in itself does not provide conclusive evidence that otherwise viable eggs were eaten. As Mrowka (1987) suggested for *Pseudocrenilabrus multicolor*, eggs may reach an advanced state of development and yet not manage to hatch out successfully.

In the Coccinellidae, reports of the extent to which viable eggs are eaten within clutches vary quite widely. On the one hand, Banks (1956) reported that viable eggs eaten within clutches amounted, on average, to 12.4% of the total clutch in *Adalia bipunctata*, 9.5% in *Coccinella septempunctata* and 5.5% in *Propylea quatuordecimpunctata*. In *Coleomegilla maculata lengi*, Pienkowski (1965) indicated that 12.7% of eggs eaten within clutches would have hatched out in the absence of cannibalism. Kaddou (1960) estimated that the proportion of viable eggs eaten was 7.8% in *Hippodamia quinquesignata* (Kirby). These authors considered that within-clutch cannibalism is an important cause of mortality.

On the other hand, Dixon (1959) stated that within-clutch cannibalism resulted in only 2.9% reduction of viable eggs in *Adalia decempunctata* (L.). Brown (1972) was of the opinion that within-clutch cannibalism in *Lioadalia flavomaculata* (De Geer) and*Cheilomenes lunata* was not of any significance because the vast majority of eggs eaten would have failed to hatch anyway in the absence of cannibalism. In line with these results, Sotherton *et al.* (1985) found that only 2% of the eggs eaten in clutches of the chrysomelid beetle, *Gastrophysa polygoni*, were viable and they concluded that cannibalism was not an important cause of mortality in this species.

The discrepancies among these results and conclusions is at least partly attributable to the methods used to estimate the proportions of viable eggs eaten. For example, Dixon (1959) and Brown (1972) removed larvae from clutches as soon as they had hatched to prevent them from feeding on the remaining unhatched eggs. All larvae resulting from eggs which at the start of hatching appeared to be fully developed were removed in this way. All other eggs, whether infertile or slow developers were left untouched. The numbers of these eggs that did subsequently hatch out were counted and were considered to be the eggs that would have been vulnerable to cannibalism in normally hatching clutches.

By this method, Brown (1972) found that only 1 of 365 *L. flavomaculata* eggs remaining in 40 clutches subsequently hatched. No larvae were obtained from 255 remaining eggs of *C. lunata.* The problem with this method, however, is that in none of these species is hatching absolutely synchronous. The larvae are therefore not only prevented from eating eggs which remain unhatched after the majority have hatched; they are also prevented from eating eggs which, although not abnormally slow developers, would nevertheless not have hatched by the time the first few larvae in the clutch had hatched. These eggs might have been vulnerable to cannibalism had the previously hatched larvae not been removed. This method could therefore lead to an underestimation of the proportion of viable eggs which are likely to be eaten in normal clutches.

At the other extreme, Banks (1956) estimated the numbers of fertile and infertile eggs that had been attacked by examining the remains of eggs after the larvae had dispersed from the clutch. Infertile eggs were identified as those with traces of yellow yolk remaining inside, while fertile eggs were assumed to be those with the dark remains of larvae within the egg shells. This method is likely to lead to an overestimation of the proportion of truly viable eggs which are eaten. As already mentioned, some eggs may reach an advanced state of development and yet not hatch out. This was shown by Dixon (1959) who found that in half of the 26 eggs which did not hatch in *A. decempunctata* the embryo had already split the chorion.

Pienkowski (1965) separated out the eggs of clutches of *C. maculata lengi* so that each egg could develop individually, uninfluenced by cannibalism. These observations allowed the numbers of non-viable, but apparently fully developed, eggs normally present in clutches to be estimated. This, in turn, could be used to estimate the numbers of viable eggs likely to have been cannibalized in normally hatching clutches. A similar method was used here to try to determine whether or not viable eggs are eaten within clutches of *Gastrophysa viridula.*

METHODS

"Field" stock female beetles were isolated in 9 cm. petri dishes lined with moistened filter paper and supplied with fresh dock leaf. Each new clutch of eggs laid was transferred on its piece of leaf to a small, 5 cm. diameter petri dish, also lined with moistened filter paper. After this, the eggs were treated in one of two different ways. Some clutches were left as they were, keeping the filter paper moist until hatching. The eggs of the other clutches were separated out using a fine moistened paint brush. Each egg was placed on a piece of dock leaf in an individual small petri dish, likewise lined with moistened filter paper. Each petri dish was labelled so as to know which clutch each egg belonged to.

On the days of expected hatching, all clutches and individual eggs were checked continuously for signs of hatching. As soon as the first egg in a clutch had hatched, including clutches of separated eggs, all the other eggs in the clutch were scored for their state of development by examining them under a binocular microscope. During the normal course of development, eggs pass through three clearly distinguishable stages. When they are laid they appear uniformly yellow, except in the cases of some infertile eggs which have a small clear portion at one of the egg's tips. Approximately 24 hours before hatching red eye pigmentation becomes visible and finally, from about 7 hours before hatching onwards, the eggs darken until the fully segmented embryo can be seen. Any yellow eggs or eggs where only the eye pigmentation is visible which are present at the start of hatching of a clutch must either be infertile eggs or ones which have developed at an abnormally slow rate.

The number of larvae that hatched out from each clutch was noted and the unseparated clutches were examined again 24 hours after the start of hatching to check whether or not the unhatched eggs had been attacked by the larvae. All unhatched eggs remaining from the separated clutches were checked daily for 7 days after hatching to determine whether they were really non-viable eggs or just abnormally slow developers.

Twenty-three complete clutches and 15 clutches in which the eggs had been separated out were examined in this way. To make sure that the groups were comparable, a two-sample t-test was used to compare the means of the clutch sizes for both groups. A chi-square analysis was then carried out to ascertain if the number of darkly-pigmented eggs that failed to hatch was independent of method of incubation. If greater than expected numbers of darkly-pigmented eggs failed to hatch when incubated in clutches, and if these eggs had been attacked by larvae, evidence would be provided that viable eggs are eaten by larvae from within their own clutches.

All manipulations of the eggs were carried out in a laminar flow cabinet.

RESULTS

Table 2.2.1. summarizes the results of the observations made during this experiment.

TABLE 2.2.1. TYPES OF EGGS PRESENT IN CLUTCHES AT THE START OF HATCHING AND THEIR HATCHING SUCCESS WHEN INCUBATED INDIVIDUALLY OR IN CLUTCHES

(Data was arcsine transformed for the analyses, but the results are presented as actual percentages in the form of mean ± S.E.)

	Incubated Individually (15 clutches)	Incubated in clutches (23 clutches)
Mean Clutch Size	41.13 ± 1.63	41.35 ± 1.59
% Darkly-Pigmented Eggs per Clutch	$98.55 \pm^{0.62}_{0.68}$	$97.94 \pm^{0.49}_{0.52}$
% Yellow Eggs per Clutch	$0.83 \pm^{0.57}_{0.42}$	$1.77 \pm^{0.51}_{0.45}$
% Red-Eye-Pigmented Eggs per clutch	$0.21 \pm^{0.22}_{0.15}$	$0.04 \pm^{0.05}_{0.03}$
% Yellow and Red-Eye-Pigmented Eggs per Clutch	$1.45 \pm^{0.80}_{0.62}$	$2.06 \pm^{0.56}_{0.49}$
% Hatch of Darkly-Pigmented Eggs per Clutch	$99.90 \pm^{0.08}_{0.13}$	$99.04 \pm^{0.73}_{0.64}$
% Hatch of Yellow and Red-Eye-Pigmented Eggs per Clutch	0	0
Total % Hatch per clutch	$98.20 \pm^{0.70}_{0.88}$	$96.03 \pm^{1.04}_{1.14}$

Hatching success of eggs incubated in isolation

The first point to make is that no red-eye-pigmented eggs or yellow eggs ever hatched out when they were individually isolated (n=17). These eggs therefore represent non-viable eggs. Together, they accounted for $1.45 \pm^{0.80}_{0.62}\%$ (n = 15) of the eggs in each of these clutches. Of the darkly-pigmented eggs allowed to develop in isolation, $99.90 \pm^{0.08}_{0.13}\%$ (n = 15) hatched out. The hatching of these eggs represents the potential viability of clutches of eggs in the absence of cannibalism and gives a mean hatching success of the total clutch of $98.20 \pm^{0.70}_{0.88}\%$.

Hatching success of eggs incubated in clutches

In clutches where the eggs remained together, all of the yellow and red-eye-pigmented eggs were eaten (n=28, a mean of $2.06 \pm^{0.56}_{0.49}\%$ per clutch for 23 clutches). However, the mean total hatch of each of these clutches was $96.03 \pm^{1.04}_{1.14}\%$. Some darkly-pigmented eggs must also have been cannibalized since all unhatched eggs had been attacked - most had had their contents completely or nearly completely eaten, but two darkly-pigmented eggs remained from a clutch of 46 which were less than half eaten.

Comparison of the hatching success of darkly-pigmented eggs when incubated in isolation and when incubated in clutches

Since some darkly-pigmented eggs did not hatch out even when cannibalism was not possible, it cannot simply be assumed that all the darkly-pigmented eggs that were cannibalized in the complete clutches were viable. A chi-square analysis was carried out to test if the number of darkly-pigmented eggs that failed to hatch was independent of experimental treatment. Only 3 out of a total of 596 darkly-pigmented eggs failed to hatch when they were incubated in isolation. Thirty out of a total of 893 darkly-pigmented eggs failed to hatch when they were incubated in clutches. A higher number of darkly-pigmented eggs therefore failed to hatch in clutches than would have been expected if the hatching success rate was independent of treatment ($X^2 = 12.93$, $p < 0.001$, d.f.=1). This was a valid comparison to make since a two-sample t-test showed that there was no significant difference between the mean clutch sizes of both groups ($t = 0.27$, $p = 0.79$, d.f.=30.9). The mean clutch size for eggs stored in isolation was 41.1 ± 1.6 (n = 15) and the mean clutch size for eggs stored in clutches was 41.4 ± 1.6 (n = 23).

Viable eggs are therefore likely to be eaten in some normal clutches of eggs, but it should be noted that in 14 out of 23 clutches (61%) where cannibalism was not excluded, all of the darkly-pigmented eggs hatched successfully.

DISCUSSION

The fact that no red-eye-pigmented eggs ever hatched out when individually isolated indicates that the presence of non-viable eggs in clutches is not solely due to the presence of infertile eggs. Even some of the darkly-pigmented eggs were non-viable. However, some viable, fully developed eggs do seem to be eaten occasionally, although by no means frequently or in great abundance. The proportion of non-viable eggs in clutches was not, therefore, the only factor limiting the extent of intra-clutch cannibalism.

Hatching of these clutches is not entirely synchronous and so it is possible that the degree of asynchrony of hatching may affect the proportion of viable eggs that earlier hatching larvae are able to attack - the more synchronous the hatching, the less chance there might be of later hatching eggs being attacked. The degree of synchrony of hatching may therefore be the limiting factor, determining the extent to which intra-clutch cannibalism can occur. This possibility will be examined in the next section. Since larvae have been shown to eat viable, related eggs, the considerations of the relatedness of cannibals to their victims and the possible individual benefits to be gained from cannibalism become quite important and will be explored in greater depth in the following chapters.

2.3. THE RELATIONSHIPS BETWEEN CLUTCH - SIZE , ASYNCHRONY OF HATCHING AND THE EXTENT OF WITHIN - CLUTCH CANNIBALISM

INTRODUCTION

When the eggs in clutches of *Gastrophysa viridula* hatch out, there may be a lag of several hours between the first egg hatching and the last. This would seemingly give the earlier hatching larvae opportunities to cannibalize many of the remaining unhatched eggs. However, on hatching, the larvae do not immediately leave their egg shells and attack unhatched eggs. The first stage of hatching is when the chorion is split in either one or two longitudinal slits. The head, thorax and legs of the larva then emerge and the larva rests with its abdomen still inside the egg shell while its body darkens and hardens. This process takes between one and three hours. After this time, the larvae move around within the egg clutch, climbing over other larvae and unhatched eggs. Only then are the unhatched eggs in danger of being eaten, and by that time other eggs will have hatched out and escaped the risk of being cannibalized. Thereafter, the chances of viable eggs being eaten will depend on the chances of their being encountered by the predatory larvae. This is likely to depend on the degree of asynchrony of hatching - the more unhatched eggs that are in a clutch after the first few larvae have left their egg shells, the greater the risk that some of them will be attacked.

Asynchrony of hatching has been suggested as a possible factor influencing the mortality of viable eggs in several invertebrate species. Banks (1956) suggested that the extent of within-clutch cannibalism was associated with the mean clutch size in each of the three species of coccinellids that he studied. In *Propylea quatuordecimpunctata* where the egg clutches contain few eggs, the proportion of darkly-pigmented eggs eaten by cannibalistic larvae was only 5.6%. In *Adalia bipunctata* and *Coccinella septempunctata* where the mean clutch sizes are larger, the proportions of darkly-pigmented eggs cannibalized was 9.5% and 12.4% respectively. Brown (1972) found that the time taken for clutches of eggs to hatch was greater in *C. lunata* than in *L. flavomaculata.* He suggested that this could account for the greater loss of fully developed eggs in *C. lunata* than in *L. flavomaculata.*

The extent of within-clutch cannibalism may therefore be linked with the degree of asynchrony of hatching within clutches and to the number of eggs within a clutch when looked at across species. Kaddou (1960), looking at these relationships within a single coccinellid species *Hippodamia quinquesignata* (Kirby), found that when the hatching period was less than one hour there was no loss of darkly-pigmented eggs. Most clutches with hatching periods of one hour or more suffered some loss of fully developed eggs. On reanalyzing his data, regression analysis showed that there was in fact a significant relationship between the hatch time and the percentage of darkly-pigmented eggs cannibalized (percentage darkly-pigmented eggs eaten = -11.385 + 0.240 hatch time, F=37.378, $p<0.001$, r.sq.=0.862, d.f.=1,13.

(percentages were arcsine transformed)). No significant relationship was found between clutch size and the percentage of fully developed eggs eaten (percentage darkly-pigmented eggs eaten = 10.932 + 0.035 clutchsize, F=0.007, p=0.933, r.sq.=0.024 d.f.=1,13).

Such effects are not restricted to insects. Baur and Baur (1986) found that by experimentally increasing the hatching asynchrony in clutches of the land snail *Arianta arbustorum*, the hatching success rate of the eggs was significantly lowered. Increased hatching asynchrony was achieved by keeping half of the eggs of clutches in a refrigerator at 9 C. for 7 days before putting them back with their sibling eggs. This cold treatment extended the incubation time of the eggs by about 4 days. Clutch size did not appear to influence the degree of hatching asynchrony (although p=0.078 by ANOVA for this relationship). The authors reported that in natural conditions, unfavourable climatic conditions such as drought or coldness may increase the hatching spread of a clutch. Because of the positioning within a clutch of eggs, some eggs will lose more water than others and some eggs may develop faster due to different temperarure exposure. Unfortunately, no data are available for the effects of clutch size on asynchrony of hatching in natural conditions for this species.

Although no reports of a significant relationship between clutch size and the percentage of viable eggs eaten have been found in the literature, observations seemed to indicate that larger clutches may take longer to hatch out and suffer a greater loss of viable eggs than smaller clutches in *Gastrophysa viridula.* These casual observations made it seem worthwhile looking more closely at the relationships between clutch size, asynchrony of hatching and the probability of viable eggs hatching successfully.

The relationships between clutch size, hatching asynchrony and the percentage hatch of viable eggs was investigated whilst carrying out the experiment described in the previous section.

METHODS

Data for the relationship between clutch size and the hatching success rate of darkly-pigmented eggs was obtained from the observations made on the 23 normally-hatching clutches described in the previous section. The course of hatching was timed in ten of these clutches. Hatching time was taken to be the time from when the first larva's head, thorax and legs emerged from its egg shell to the emergence of the last larva in the clutch. Regression analysis and multiple regression analysis were used to investigate the relationships between clutch size, hatching time and the percentage of viable eggs hatching in each clutch. For the analyses, darkly-pigmented eggs were assumed to be viable eggs. In practice, 99.90% of darkly-pigmented eggs are in fact viable (see previous section). All percentages were arcsine transformed for the analyses.

RESULTS

Relationship between clutch size and the hatching success of viable eggs

The percentage of darkly-pigmented eggs that hatched in each of the 23 clutches is plotted against clutch size in Figure 2.3.1. Regression analysis showed that the relationship between clutch size and the hatching success of darkly-pigmented eggs was significant (% hatch darkly-pigmented eggs= 104.202 - 0.479 clutch size. F=4.866, p=0.04, r. sq.=18.4% d.f.=1,21).

Relationship between hatch time and the hatching success of viable eggs

Using the data from the ten clutches in which the hatch time was also recorded, a significant relationship was found between hatch time and the percentage hatch of darkly-pigmented eggs. The greater the hatch time, the smaller the proportion of viable eggs to hatch (% hatch of darkly-pigmented eggs = 99.2 - 0.067 hatch time, F=15.03, p<0.005, r.sq.=65.3%, d.f.=1,8. Figure 2.3.2.). The hatch time ranged from 163 minutes to 532 minutes with a mean of 291.4 ± 40.1minutes (n = 10).

FIGURE 2.3.1. **THE RELATIONSHIP BETWEEN PERCENTAGE HATCH OF VIABLE EGGS AND CLUTCH SIZE**

The regression line is shown for clutch sizes of 38-51 eggs.

The equation of the line is % Hatch = 137.21-1.23 clutch size

(see Table 2.3.1)

+ = 1 data point

◆ = 2 data points

⊕ = 3 data points

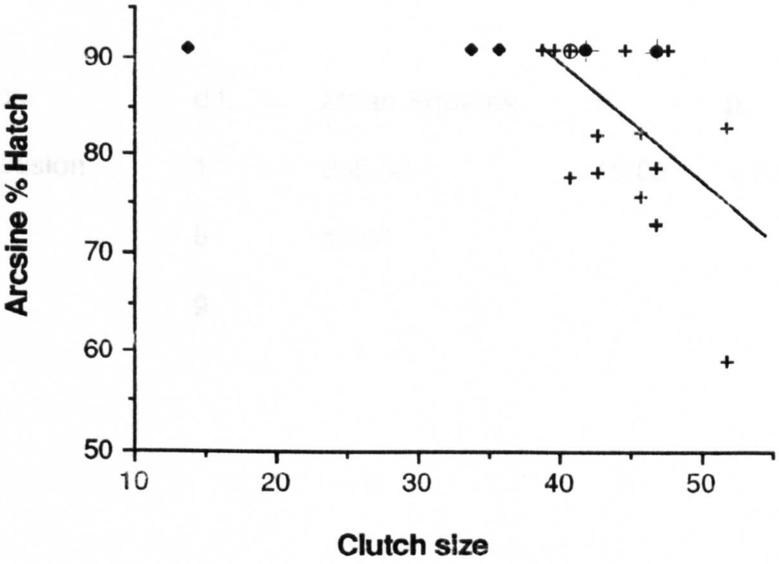
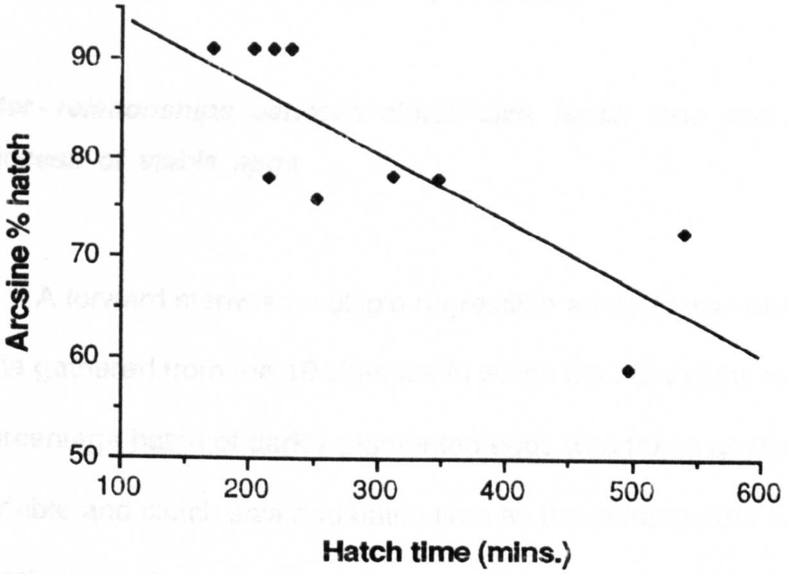

Arcsine % Hatch
90
80
70
60
50
10
20
30
40
50
Clutch size

FIGURE 2.3.2. THE RELATIONSHIP BETWEEN PERCENTAGE HATCH OF VIABLE EGGS AND HATCH TIME

The equation of the regression line is % Hatch = 99.2 - 0.07 Hatch Time.

ANALYSIS OF VARIANCE

Source	d.f.	Mean Squares	F	p	r. sq.
Regression	1	655.32	15.03	< 0.005	65.3%
Error	8	43.61			
Total	9				


Arcsine % hatch
90
80
70
60
50
100
200
300
400
500
600
Hatch time (mins.)

Relationship between clutch size and hatch time

A significant relationship was also found between the clutch size and the hatch time. The greater the clutch size, the greater the time required for all the larvae in the clutch to hatch out (hatch time = -838 + 26.6 clutch size, F=15.42, p<0.005, r.sq.=65.8%, d.f.=1,8. Figure 2.3.3.).

Inter - relationships between clutch size, hatch time and the hatching success of viable eggs

A forward stepwise multiple regression analysis was performed on the data gathered from the 10 clutches in which the hatch time was measured. Percentage hatch of darkly-pigmented eggs was taken as the dependent variable and clutch size and hatch time as the independent variables. Only clutch size entered the regression equation - (% hatch of darkly-pigmented eggs = 180.01-2.38 clutch size. F = 21.14, p=0.0018 r.sq. = 72.5%, d.f.=1,8). The primary factor affecting the hatching success rate of viable eggs in these ten clutches was therefore clutch size.

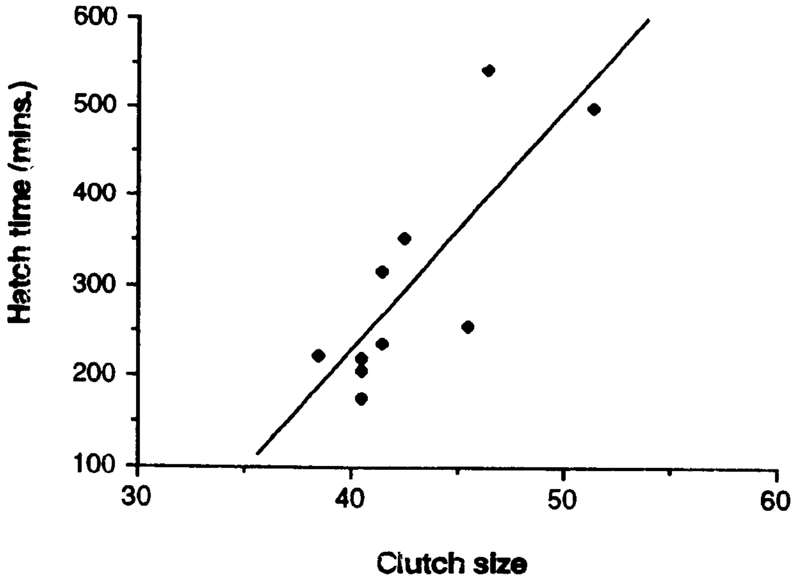
A closer investigation of Figure 2.3.1. reveals that the relationship between percentage hatch of viable eggs and clutch size may not be a straightforward negative one over the entire range of clutch sizes. Clutches with 39 or fewer eggs (5 clutches) did not suffer the loss of any viable eggs. This data suggests the possible existence of a shoulder point below which all

FIGURE 2.3.3. THE RELATIONSHIP BETWEEN HATCH TIME AND CLUTCH SIZE

The regression equation is Hatch Time = -838 + 26.6 Clutch Size

ANALYSIS OF VARIANCE

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	95328	15.42	<0.005	65.8%
Error	8	6182			
Total	9				


600
500
400
300
200
100
Hatch time (mins.)
30
40
50
60
Clutch size

viable eggs generally hatch successfully and above which the risk of viable eggs being cannibalized increases with increasing clutch size.

Such a pattern in the relationship between hatching success rate of viable eggs and clutch size is a reasonable expectation. It takes between one and three hours from the first stage of hatching of the first larva until other eggs in the clutch become vulnerable to cannibalism - when the first larva starts to move around within the clutch. During this time a certain number of eggs will have successfully hatched. This recalls the findings of Kaddou (1960) in his studies of the coccinellid species *H. quinquesignata.* He found that when the hatching period was less than one hour there was no loss of darkly-pigmented eggs and that most clutches with hatching periods of one hour or more suffered some loss of fully developed eggs. Given that in *G. viridula* it has been found that there is a positive relationship between hatch time and clutch size (see Figure 2.3.3.) it is likely that clutches below a certain size will generally suffer no or little cannibalism of viable eggs. Above such a clutch size, the risks of cannibalism of viable eggs are likely to increase. This is because the number of unhatched eggs remaining in a clutch when the first larvae leave their egg-shells and become cannibalistic is likely to increase with increasing clutch size, thus increasing the probability of larvae having left their egg-shells encountering unhatched viable eggs.

A regression analysis was carried out on the results of percentage hatch of viable eggs and clutch size in which only data from clutches with 40 or more eggs was included. This was the range of clutch sizes over which there appeared to be a negative relationship between these two variables (see Figure 2.3.1). The regression produced a significant result (% hatch = 136.655 - 1.222 clutch size. F=4.951, p=0.042, r.sq.=23.62, d.f.=1,16). The equation is in the form % hatch = k - a n, where k and a are constants and n is clutch size.

A shoulder clutch size, n_S, that results in 100% hatching success of viable eggs can be predicted from this regression. Percentage hatch takes the value 90, since the data was arcsine transformed, then $n_S = (k - 90) / a$. This gives a shoulder value of 38.2. The regression analysis was therefore repeated using data from clutches with 38 or more eggs and also using data from clutches with 39 or more eggs to check the robustness of the result. Both regressions predicted a shoulder clutch size of 38.3. These results are presented in Table 2.3.1.

However, the calculation of a shoulder clutch size from the present data should be regarded as being of an extremely provisional nature. There is a lack of data from clutches containing low numbers of eggs (see Figure 2.3.1.). However, artificially reduced clutches of 16 eggs each were obtained during an experiment designed to investigate the extent to which eggs of different ages were cannibalized by first-instar larvae (see Chapter 4). It was

TABLE 2.3.1. REGRESSION ANALYSES TO TEST THE RELATIONSHIP BETWEEN PERCENTAGE HATCH OF VIABLE EGGS AND CLUTCH SIZE

The results of regression analyses in which different clutch sizes (n) were included, along with the clutch size that maximizes the number of hatched larvae per clutch (n_*) and the "shoulder" clutch (n_S) size that they predict.

Clutch size	REGRESSION EQUATION	n_*	n_S
13-51	% Hatch = 104.20 - 0.48n	108.8	29.7

ANALYSIS OF VARIANCE

Source	d.f.	Mean Squares	F	p	r. sq.
Regression	1	293.23	4.87	0.04	18.84%
Error	21	60.26			
Total	22				

	REGRESSION EQUATION	n_*	n_S
38-51	% Hatch = 137.21 - 1.23n	55.5	38.26

ANALYSIS OF VARIANCE

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	412.35	7.15	0.02	28.41%
Error	18	57.65			
Total	19				

	REGRESSION EQUATION	n_*	n_S
39-51	% Hatch = 137.58 - 1.24n	55.4	38.3

ANALYSIS OF VARIANCE

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	368.67	6.04	0.03	26.21%
Error	17	61.03			
Total	18				

	REGRESSION EQUATION	n_*	n_S
40-51	% Hatch = 136.66 - 1.22n	55.9	38.2

ANALYSIS OF VARIANCE

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	320.83	6.95	0.04	23.62%
Error	16	64.72			
Total	17				

necessary to know how many of the 16 eggs hatched out as the number of larvae in each trial could have affected the number of eggs eaten and had to be controlled for in the analysis. The developmental state of any remaining eggs in each clutch was recorded - whether yellow, with red eye pigmentation only or darkly-pigmented. The percentage of darkly-pigmented eggs that had hatched out could therefore be calculated and was $99.98 \pm^{0.01}_{0.02}$ % (n=86).

Given that a small proportion of darkly-pigmented eggs are not viable anyway, it is almost certain that 100% of the viable eggs hatched out in these reduced clutches. This supports, to some extent, the view that small clutches escape cannibalism of viable eggs. However, in order to verify the existence of a shoulder clutch size, it would be necessary to obtain data from a full range of clutch sizes. This could be achieved by experimental manipulation. For example, females could be interrupted at varying stages of their laying in order to obtain clutches containing from two eggs through to the highest numbers of eggs per clutch obtained by uninterrupted laying.

Such data would be sufficient to verify or refute the existence of a shoulder clutch size. If a shoulder clutch size was found to exist, with sufficient data across a complete range of clutch sizes, its value could be calculated with much greater confidence than the presently available data allows.

Even though in clutches with more than 38 eggs there was an increasing risk of darkly-pigmented eggs failing to hatch with increasing clutch size, it is not valid to ascribe this to the effects of cannibalism of viable eggs without first ruling out the possibility that with greater clutch sizes a greater proportion of darkly-pigmented eggs do not hatch out anyway. This was tested by regression analysis of the percentage hatch of darkly-pigmented eggs on clutch size when the eggs were reared in isolation to prevent cannibalism. The 15 clutches reared in this way as described in the previous section were used for this analysis. Under these conditions, there was no significant relationship between the clutch size and the percentage of darkly-pigmented eggs that successfully hatched out (Figure 2.3.4.). It is therefore valid to conclude that cannibalism of viable eggs increased with increasing clutch size when the clutches contained 38 or more eggs.

DISCUSSION

If it is provisionally assumed that, as suggested by the data, the relationship between clutch size and rate of successful hatching of viable eggs may be one of complete hatching success in clutches up to a certain shoulder size and thereafter one of a decreasing rate of hatching success with increasing clutch size, the clutch size which would maximize the number of successfully hatching larvae per clutch can be calculated.

The method used is based on that which has been developed by authors

FIGURE 2.3.4. **THE RELATIONSHIP BETWEEN PERCENTAGE HATCH OF VIABLE EGGS AND CLUTCH SIZE WHEN THE EGGS ARE INCUBATED INDIVIDUALLY**

+ = 1 data point

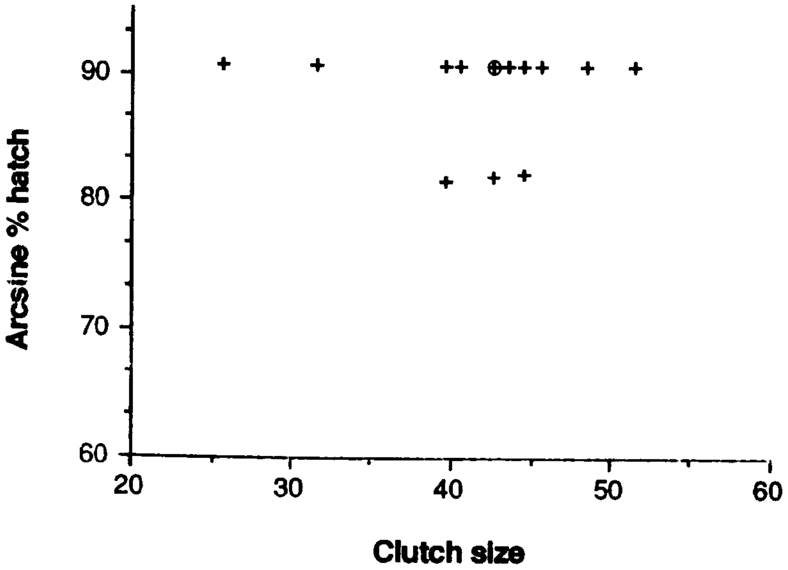
⊕ = 3 data points

The regression equation was not significant

(Percentage Hatch = 89.14 - 0.02 Clutch Size)

<u>ANALYSIS OF VARIANCE</u>

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	0.29	0.02	0.89	0.15%
Error	13	14.94			
Total	14				


Arcsine % hatch
90
80
70
60
20
30
40
50
60
Clutch size

in recent papers to calculate the "Lack" clutch size in invertebrates (e.g. Parker and Courtney, 1984; Charnov and Skinner, 1984, 1985; Begon and Parker, 1986; Parker and Begon, 1986 and Godfray, 1986, 1987). Originally, Lack stimulated interest in the problem of clutch size in birds (Lack, 1947, 1954). He suggested that clutch size evolved to maximize the survivorship of the parents' offspring to adulthood.

This idea has also formed the basis of many of the more recent investigations of clutch size in invertebrates. The basis of the models developed is the assumption that the probability of survival to adulthood of larvae in a clutch will decline with increasing clutch size. To find the clutch size that maximizes the number of larvae surviving to adulthood the product of n and $s(n)$ is maximized, where $s(n)$ describes the probability of larval survival when n eggs are laid in a clutch (cf. Parker and Courtney, 1984). The same method can be used to calculate the clutch size which would maximize the number of successfully hatching larvae per clutch, n^*, but in this case $s(n)$ describes the probability of a viable egg hatching out successfully when n eggs are laid in the clutch. The calculation is as follows:-

$$\frac{d}{dn}\,[n \,.\, s(n)] = 0, \quad n_* = -\,s(n_*)/s'(n_*).$$

Assuming that $s(n)$ declines linearly with n, then

$$s(n) = k - an \quad \text{and} \quad n_* = k/2a.$$

Taking the regression equation that included clutch sizes of 38-51 eggs

(% hatch = 137-1.23 clutch size. See Table 2.3.1) ,

$n_* = 137/2.46 = 55.7$

The other significant regression equations including clutch sizes above 38 eggs give similar results : $n_* = 55.6$ when clutch sizes of 39-51 are included in the regression and $n_* = 56.1$ when clutch sizes of 40-51 are considered.

It should be noted that the clutch size which maximizes the number of successfully hatching larvae per clutch is not the same as the "Lack" clutch size which maximizes the fitness of a female on a per clutch basis. The fitness of a female (*wf*)can be calculated from the product of the number of offspring produced and their mean fitness (Skinner, 1985). For example, the fitness through offspring that a female can realize by laying a given clutch size (restricting the calculation to female offspring) was calculated as follows by Charnov and Skinner, 1985:-

wf = (clutch size) x (proportion of offspring surviving to adulthood) x (lifetime egg production for adult offspring of the size produced from such a clutch).

According to Lack's hypothesis, natural selection would favour the clutch size which maximizes *wf*. Lifetime egg production for adult offspring of a given size was included in the calculation because in some insects, such as parasitic hymenoptera, crowding of immatures may lead to the production of small adults, and it has been shown that adult body size may have large effects on egg production (cf. Charnov *et al*, 1981). However, in the case of *Gastrophysa viridula*, no evidence was obtained from laboratory observations to suggest that lifetime egg production was affected by adult size (see Chapter 5). If lifetime egg production was found to be similarly unaffected by adult size under field conditions, the calculation of the "Lack" clutch size would entail maximizing the product of clutch size and the proportion of offspring surviving to adulthood.

The clutch size that maximizes the number of successfully hatching larvae per clutch would only be analogous to the "Lack" clutch size if, after hatching, it was found that a negative correlation between the number of larvae successfully hatched from a clutch and their average survivorship to adulthood or some other aspect of their fitness did not exist.

Such a negative correlation could be caused by competition for food among larvae. Most models of insect clutch size have been developed with respect to insect parasitoids, or insects with similar life histories, in which females lay their eggs in larval food resources of limited carrying capacity and the decline in fitnes is due to the increasing competition among the larval offspring from a single clutch with increasing clutch size. The situation in *G. viridula* is not quite the same.

On an experimental plot of *Rumex obtusifolius*, Smith and Whittaker (1980b) found that leaf area consumption by the population of *G. viridula* was never greater than 5% of the standing crop of foliage. Abundance curves for this population (Smith and Whittaker, 1980a) show that during one generation of the beetles there were peak mean numbers per plant of 2 adults, 8 clutches of eggs, 47 first instar larvae, 18 second instar larvae and 13 third instar larvae. Obviously, the larvae resulting from a single clutch of eggs are unlikely to significantly deplete their food source, particularly since larvae are able to move between different leaves on a particular plant and between different plants within a clump. However, if many clutches of eggs are laid on all the plants within a clump it is possible that larvae could deplete their food supply to such an extent that competition between the larvae could become important.

Predation is another possible factor which could cause a negative correlation between the number of larvae hatched from a clutch and their average survivorship to adulthood. After hatching, first instar larvae from a particular clutch remain in a group. If larger clutches or larger groups of larvae are prone to higher rates of predation than smaller groups this would result in a negative relationship between the number of hatched larvae and their average survivorship to adulthod.

There is also the possibility that intra-clutch cannibalism may result in the clutch size which maximizes the number of larvae hatching per clutch not being analogous to the "Lack" clutch size. Evidence presented in Chapter 4 suggests that older eggs are in some ways protected against cannibalism and since eggs have been shown to be toxic against potential predators such as ants, there is some reason to suspect that the protective mechanism against canibalism may also be one of chemical defence. If older eggs are toxic it would be detrimental to the fitness of larvae (and hence to the fitness of their mother) to eat eggs that are close to hatching in their own clutches. If such were the case, the clutch size which maximizes the number of larvae successfully hatching from a clutch might not correspond to the "Lack" clutch size which maximizes a female's fitness per clutch.

From the available data, it is hypothesized that a "shoulder" clutch size may exist below which all viable eggs in a clutch will generally hatch out and above which there is a negative relationship betweeen the probability of hatching and clutch size. A shoulder value of 38 eggs has been provisionally calculated. Likewise, using the available data, the clutch size which would maximize the number of successfully hatching larvae per clutch has been provisionally calculated as 56 eggs.

It must be stressed, however, that neither a shoulder clutch size or clutch size that is predicted to maximize the number of hatched larvae per clutch should be taken as definitive. More data is required across a fuller range of clutch sizes before the existence of a shoulder clutch size can be either verified or refuted and, if such a shoulder size does exist, before its value and that of the clutch size that maximizes the number of hatched larvae per clutch can be calculated with any great confidence. Nevertheless, the provisional calculation of these values poses questions that would be interesting to investigate in the future.

The mean clutch size laid by females was 41.35 ± 1.6 (n=23). With a fuller set of data, it would be interesting to find out if females do, on average, lay clutch sizes which are closer to a value which minimizes the risks of intra-clutch canibalism than to the clutch size which would maximize the number of hatched larvae per clutch.

The rate of cannibalism of viable eggs in normal clutches is certainly very low. It was found that a mean of 99% of viable eggs hatched from normal clutches (seeTable 2.2.1) and that in 61% of clutches there was no cannibalism of viable eggs at all (see previous section). This suggests the possibility that the limitation of intra-clutch canibalism may have been a factor which has influenced the evolution of clutch size in *G. viridula*. It would therefore seem worthwhile to investigate the fitness effects on larvae of eating viable eggs from within their own clutches.

However, to investigate more fully the factors which may have been involved in the evolution of clutch size in this insect it would be necessary to try to evaluate the "Lack" clutch size, investigating the effects not only of intra-clutch cannibalism, but also of predation and competition on the survival of larvae from different-sized clutches to adulthood and on their subsequent reproductive output. Factors influencing females' fitnesses over their entire lifetimes, rather than on a per clutch basis should also be investigated. The problem of the evolution of an optimal clutch size in *G. viridula* is discussed further in Chapter 7.

CHAPTER 3

SPERM COMPETITION IN *G. VIRIDULA* AND THE RELATEDNESS BETWEEN CANNIBALS AND THEIR VICTIMS

Outline of Chapter 3

In this chapter the possible mechanism of sperm competition in *G. viridula* is explored. The importance of the probable relatedness between the eggs of a particular clutch for the evolution of cannibalistic behaviour is discussed.

INTRODUCTION

In trying to understand the conditions that may have selected for the evolution of cannibalism in *Gastrophysa viridula*, it is necessary to have some knowledge of both the benefits that may accrue to cannibals and the relatedness between cannibals and their victims. With regard to selfish behaviour in general, Hamilton (1964a,b) stated that genes causing this type of behaviour will not advance in frequency if the behaviour results in an increase in fitness that is not sufficient to compensate for the loss of fitness in close relatives. This is due to the likelihood of close relatives carrying replicas of the same genes. The particular conditions that must be fulfilled for positive selection of the causative gene(s) to occur are given in Hamilton's (1964a) criterion, $-k < 1/r$. Here, k is the change in fitness of the victim divided by the gain in fitness of the actor and r is the coefficient of relatedness between the two individuals.

The possible benefits that cannibals may gain from eating their conspecifics is the subject of Chapter 5. In the present chapter, the importance of the relatedness between cannibals and their victims will be considered. The coefficient of relatedness between full-siblings is 0.5 and between half-siblings is 0.25. This difference between the coefficients of relatedness is quite considerable. The conditions that would select for cannibalism of full-siblings are therefore much more stringent than those that would favour cannibalism of half-siblings. In the case of full-siblings, cannibalism would only be selected for if the personal gain to the cannibal was more than half the loss of fitness in the victim. If the interacting individuals are half-siblings, cannibalism would evolve if the gain to the cannibal is more than a quarter times the loss of fitness in the victim.

Since it has been established that cannibalism of viable eggs sometimes takes place within *G. viridula* clutches, it is important to know the probable relatedness between the larvae and their egg victims in these situations. If each female mated with only one male, the eggs in any particular clutch would be full-siblings. However, the females of many insect species mate more than once with different males (Parker, 1970a; Walker, 1980; Gwynne,1984). Unlike the sperm of many other animals, insect sperm can be stored by the female and remain viable for considerable periods of time. This situation creates the potential for the sperm of more than one male to compete for the fertilization of the female's ova (Parker, 1970a; see Davey, 1985).

In multiply-mating clutch-laying species, the relatedness between the eggs of a particular clutch will depend on the way in which the females use the sperm they receive from different males. For example, even if females mate with more than one male, they may not remate until their current sperm supply is almost completely depleted. This appears to be the case in the cockroach, *Blatella germanica*, (Cochran, 1979) and in *Drosophila melanogaster* (Letsinger and Gromko, 1985). In these species, there will be little opportunity for direct sperm competition and most of the eggs within a clutch will be full-siblings. Similarly, if the sperm of the last male to mate with a female lie nearest to the site of fertilization, this sperm will predominate at fertilization and again, most of the eggs within a clutch may be full-siblings.

For example, Siva-Jothy (1984) found that males of the libellulid dragonfly *Crocothemis erythraea* reposition the sperm of previous males so that their own sperm will have precedence at fertilization. Among the odonata, males commonly remove the sperm of earlier males before inseminating with their own (Waage1979 a, b; 1984). The degree of relatedness betwen the eggs produced by a female will then depend on how much of the earlier males' sperm is removed. Waage (1984) reported that in *Calopteryx dimidiata*, males remove almost all (ca. 98%) of the sperm of previous males. This would result in the females' offspring being mostly full-siblings. Higher proportions of half-siblings could be present in females' clutches if sperm removal or displacement was not so complete.

In several species of insects, the sperm of the last male to mate mixes with the sperm of earlier males. Unless there is also some sperm removal or repositioning, the last male will fertilize a proportion of the female's ova corresponding to the proportion of the total sperm received by a female that his own sperm represents in the female's spermatheca (Parker,1970a; Walker,1980). A sperm mixing system could therefore result in both half-siblings and full-siblings being present in females' clutches. The relative proportions of half-siblings and full-siblings present in a clutch will depend on the number of males the female has mated with before the eggs in the clutch are fertilized, and the number of sperm from each male present in the spermatheca. Sperm mixing has been shown to occur, for example, in crickets (Backus and Cade, 1986; Sakaluk, 1986 and Simmons,1987) and in the cockroach, *Diploptera punctata* (Woodhead, 1985).

Sperm mixing has also been found in two species of chrysomelid beetles. Although Shimizu and Fujiyama (1986) stated that sperm displacement was found in *Chrysolina aurichalcea* by Fujiyama and Watanabe (unpublished), the evidence they presented indicates that sperm mixing occurs in this chrysomelid beetle. They reported that when two males mated for equal durations with females, both males were equally represented in the subsequent offspring. Dickinson (1986), reported that sperm mixing occurred in the milkweed leaf beetle, *Labidomera clivicollis clivicollis*. In double matings, the proportions of eggs fertilized by each male was not significantly different from that which would be expected if paternity was determined by sperm mixing, correcting for the time of mating of each male.

However, in a more recent paper, Dickinson (1988) has presented evidence that indicates that the mechanism of sperm predominance involves time-dependent sperm removal and replacement as well as subsequent sperm mixing. In double matings, where the duration of each mating was 15 hours, the mean proportion of offspring attributable to the second males did not differ significantly from 0.5. But the mean proportion of offspring attributable to second males was significantly greater than 0.5 when the matings were of 45 hours each, or if they were of 15 hours each with a gap of 5 days between the two matings. The number of sperm present in females' reproductive tracts after a single 45 hour mating did not differ significantly from the number of sperm in the reproductive tracts of females mated to two males for 45 hours each. This circumstantial evidence supports the hypothesis that males achieve sperm precedence by a combination of flushing out sperm from previous males and sperm mixing. This is the same sort of mechanism that was suggested by Parker (1970b) to account for the pattern of sperm precedence in the dung fly, *Scatophaga stercoraria.*

Gastrophysa viridula females have been observed in the laboratory to mate with more than one male. The potential, at least, for sperm competition is therefore present in this species. A sperm competition experiment was carried out to investigate the likelihood of full- and half- siblings being present in females' clutches.

METHODS

The beetles used in this experiment were from the "Field" laboratory stock. Final instar larvae were reared individually through to the adult stage in small petri dishes. This ensured that virgin females were obtained.

To investigate the probable relatedness of eggs within clutches of multiply-mated females, the simplest case of double mating was examined. The technique of irradiating one of the males before mating was used to estimate the proportion of fertilized eggs in clutches attributable to each of the two males (see Parker 1970b). Irradiation causes sperm to carry high levels of dominant lethal mutations. Although the sperm remain capable of fertilizing ova, varying degrees of mortality in the early embryonic stages will result, depending on the level of irradiation.

A trial experiment was undertaken to determine the most appropriate level of irradiation to use. X-ray irradiation was from a Philips SL75/5 linear accelerator. Irradiation may have adverse effects on the mating behaviour of the males and on the motility and numbers of sperm produced (Zimmering and Fowler,1966; Parker, 1970b; Parker and Smith, 1975). For this reason, it is judicious to choose the minimum level of irradiation that causes the desired degree of mortality in the developing embryos. The level of irradiation chosen was 70 Gray (dose rate 10 Gray / minute). At this level, very high levels of mortality were induced, without causing complete mortality.

Females were placed in small 5-cm. diameter petri dishes lined with moistened filter paper and supplied with a piece of dock leaf. Although the females were virgins, they had already been laying eggs for between 4 and 7 days. They were divided into four groups to receive different combinations of normal or irradiated males as mates. The four groups were NN (two normal males), RR (two irradiated males), NR (one normal followed by an irradiated male) and RN (one irradiated followed by a normal male).

It had previously been observed that males will mount, dismount and remount the same female intermittently. During the experiment, the total time spent in the mounted position by each male was recorded until the male did not remount within half an hour of dismounting. It should be noted that actual genital contact did not necessarily take place during the whole of the mounted period. No attempt was made to record the time of genital contact in mating pairs. The second male of each pair was presented to the female within ten minutes of removing the first male. Any females that had not mated with a male within 12 hours of its introduction were excluded from the experiment. Double matings were achieved in 8 NN females, 7RR females, 9 NR females and 10 RN females.

When the matings were completed, each female was transferred to a 9 cm. diameter petri dish lined with moistened filter paper and supplied with a fresh piece of dock leaf. The petri dishes were checked twice daily for eggs and the pieces of dock leaf were changed every day or whenever a new clutch of eggs was laid, whichever was the most frequent. When a new clutch of eggs was found it was transferred, on the piece of dock leaf, to a small petri dish lined with moistened filter paper. The number of eggs per clutch and the subsequent numbers of larvae that hatched were recorded for the first ten clutches laid by each female, except in cases where fewer than ten clutches were laid. The percentage of eggs that hatched in each of these clutches was calculated. The transfer and counting of eggs was done in a laminar flow cabinet to avoid fungal infection.

The method of Boorman and Parker (1976) was used to estimate the proportion of eggs fertilized by the second male (P_2) in each of the clutches laid by NR or RN females. The proportion of eggs that hatch from NN females is designated p. The proportion $1-p$ that fail to hatch may be due to ova not having been fertilized, fatal abnormalities in the development of embryos and / or cannibalism. Irradiation may result in a small proportion (z) of eggs successfully hatching after RR matings. The proportion of eggs fertilized by an R male (P_R) when a proportion x eggs hatch after a mixed double mating is then given by:-

$$P_R = \left(1 - \frac{x}{p}\right) + \left(\frac{z}{p} \times \frac{1-x/p}{1-z/p}\right)$$

The first part enclosed by brackets on the R.H.S. of this equation corresponds to the depression in fertility caused by the sperm from the R male. The second part enclosed by brackets is the expected proportion of hatching eggs derived from the R sperm. In the case of NR matings $P_2 = P_R$ and for RN matings $P_2 = 1-P_R$. The mean p and z values were calculated individually for all 1st, 2nd, 3rd,.......10th clutches of the 8 NN and 7 RR females. In calculating the P_2 of a particular clutch of an NR or RN female, the appropriate p and z value was used, according to whether the clutch was the 1st, 2nd, 3rd etc. laid by the female.

Regression analysis was used to investigate any changes that may have occurred in the P_2 values over successive clutches for individual females. Two models were constructed to describe the possible mechanisms of sperm competition in *Gastrophysa viridula*. Regression analysis was used to test how well the data fitted the predictions of the models. A paired-sample t-test was used to compare the duration of first and second matings in all NN, RR, NR and RN females.

RESULTS

Changes in P_2 values over successive clutches

The mean proportions of eggs hatching in successive clutches of NN and RR females are shown in Table 3.1.a. The proportions of eggs fertilized by the second male, P_2, in succesive clutches of NR and RN females are shown in Table 3.1.b. One of the NR females did not produce any clutches after mating, leaving only 8 NR females in the experiment.

The observed proportions of eggs hatching (x) were sometimes higher than the mean p value or lower than the mean z value used in the calculation of P_2 values. This resulted in estimated P_2 values either slightly greater than 1 or slightly less than 0. For statistical analysis, such values were corrected to 1 and 0 respectively. P_2 values were arcsine transformed for regression analysis.

Regression analysis showed no obvious trend in the changes of P_2 values in successive clutches. Ten of the females showed a negative trend in their P_2 values, two of which were significant. Seven of the females showed a positive trend in their P_2 values with increasing clutch order, one of which was significant (see Table 3.1.b).

TABLE 3.1. THE PROPORTIONS OF EGGS HATCHED IN NN AND RR FEMALES' CLUTCHES, THE P_2 VALUES OF SUCCESSIVE CLUTCHES OF NR AND RN FEMALES AND THE DURATIONS OF FIRST AND SECOND MATINGS WITH NR AND RN FEMALES

a) THE MEAN PROPORTION OF EGGS HATCHED (*p* and *z*) OF ALL 1st, 2nd,......10th CLUTCHES OF 8 NN FEMALES AND 7 RR FEMALES RESPECTIVELY

The means were calculated from arcsine transformed proportions, but have been reconverted to actual proportions.

Clutches	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Mean *p*	.985	.985	.951	.964	.944	.966	.948	.937	.960	.947
Mean *z*	.010	.007	.015	.011	.002	.002	.002	.007	.006	.0003

b) P_2 VALUES OF SUCCESSIVE CLUTCHES OF NR AND RN FEMALES WITH THE RATIOS OF DURATION OF FIRST TO SECOND MATINGS (*a*/*b*)

The P_2 values were calculated from the proportions of eggs hatching in each clutch and the appropriate *p* and *z* values (see methods for details).

i) NR Females					P_2 for each clutch						Regression on clutch order
a/*b*	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	(b± S.E.)
.02	1.01	.96	.96	1.01	1.00	.98	.95	.98	.93	1.0	N.S.
.05	1.01	.95	.91	1.01	.97	1.0	1.0	.93	.98	.95	N.S.
.22	.93	1.01	.94	0.94	.98	.90	.98	.91	.98	.87	N.S.
.22	.99	.98	1.02	.99	.98	.93	1.00	1.01	1.01	.98	N.S.
.74	1.01	.98	1.02	.99	1.00	.98	1.00	.98	1.01	-	N.S.
.92	.93	.80	.86	.70	.37	.56	.47	.48	.62	.78	N.S.
1.36	.37	.38	.25	.26	.17	.56	.46	-	-	-	N.S.
13.83	0.0	-.01	-.05	-.04	-.06	-.03	-.03	-.01	-.04	0.0	N.S.
ii) RN Females											
.11	.80	.92	.95	1.01	.98	.86	1.03	1.07	1.04	1.01	2.43 ± .26*
.19	1.0	.99	1.03	1.04	1.03	.76	-	-	-	-	N.S.
.19	.95	.99	.86	1.04	1.04	.99	1.05	-	-	-	N.S.
.29	.98	.84	.74	.83	.95	.92	.94	.96	.84	.99	N.S.
.40	-.01	-.01	0.0	.02	0.0	0.0	0.0	-.01	-.01	0.0	N.S. ß
.53	.66	.54	.68	.73	.74	.71	.73	.79	.63	.74	N.S.
.64	.73	.62	.60	.53	.47	.25	.30	.46	.19	.12	-3.84 ±.19**
.89	.48	.66	.74	.64	.72	.56	.55	.65	.52	.57	N.S.
1.37	.97	.99	.85	.90	.94	.87	.92	.83	.68	.87	-2.01 ± .20*
2.64	.74	.34	.36	-	-	-	-	-	-	-	N.S.

* = $p < 0.05$, ** = $p < 0.001$.

ß = results from this female were abnormal and were not included in regression analysis to test the predictions of Models 1 and 2.

Models to examine the mechanism of sperm competition

Two models to describe the mechanism of sperm competition in *G. viridula* were considered. Both models assume complete sperm mixing. The first model assumes linear sperm transfer with time. The number of sperm transferred by a male is therefore directly proportional to the mating duration. In double matings, if *a* is the duration of the first mating and *b* is the duration of the second mating, then

$$P_2 = b / (a + b)$$

$$\text{and} \qquad 1/P_2 = (a/b) + 1.$$

If the assumptions of the model hold true, a regression of $1/P_2$ against a/b would give a straight line with a Y-intercept of 1 and a regression coefficient, or slope, of 1.

The second model assumes complete sperm mixing, but instead of linear sperm transfer, it assumes diminishing sperm numbers transferred with increasing time. There are several ways in which this relationship could be modelled. One suitable function to describe the number of sperm transferred (S) with time is:-

$$S = \log_e(k.t + e) - 1,$$

where k is a parameter and t is the duration of the mating. It fulfills the criteria of diminishing numbers of sperm transferred with increasing time and matings of time zero producing zero sperm transfer. The relationship described by this function is shown in Figure 3.1.

For the case of double matings, where *a* is the duration of the first mating and *b* is the duration of the second mating as before, then

$$P_2 = \frac{\log_e(k.b + e) - 1}{[\log_e(k.a + e) - 1] + [\log_e(k.b + e) - 1]}$$

and

$$1/P_2 = \frac{\log_e(k.a + e) - 1}{\log_e(k.b + e) - 1} + 1.$$

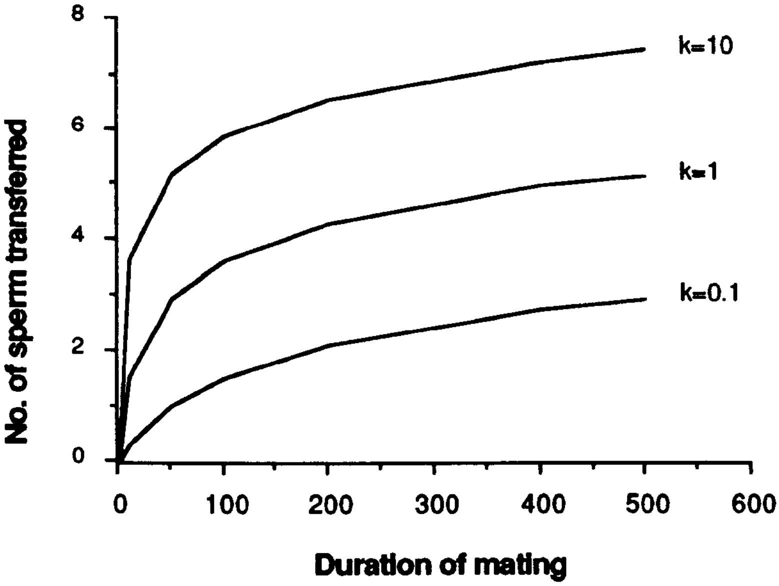
If the assumptions of the second model hold true, regression analysis of the latter relationship would again give a straight line with a Y-intercept of 1 and a regression coefficient of 1.

The P_2 values and the durations of the first and second matings for NR and RN females (see Table 3.1.b) were used to test how closely the results fit the expectations of the two models. Using the Kolmogorov-Smirnov goodness of fit test, it was found that the $1/P_2$ values did not differ significantly

FIGURE 3.1. **THE ASSUMED RELATIONSHIP FOR MODEL 2 BETWEEN THE NUMBER OF SPERM TRANSFERRED BY MALES TO FEMALES AND THE DURATION OF MATING**

The assumed relationship is Number of sperm transferred = $\log_e(k.t + e) - 1$,

where t = the duration of the mating and k = a parameter.



8
6
4
2
0
No. of sperm transferred
k=10
k=1
k=0.1
0
100
200
300
400
500
600
Duration of mating

from the normal distribution. These values were not therefore arcsine transformed. Cases where the P_2 value was zero could not be entered in the regression since $1/P_2$ in these cases does not exist. One of the RN females (marked with β in Table 3.1) had mated more than twice as long with the normal male than with the irradiated male. The P_2 values from her clutches, however, indicated that the normal male had not fertilized any of her eggs. This result was so obviously an abnormal result that it was excluded from the regression analyses. The male may have been infertile or may not have transferred any sperm to the female.

For the first model, a regression was carried out, taking $1/P_2$ as the dependent variable and a/b as the independent variable. The results of this regression are presented in Table 3.2. The regression equation is $1/P_2 = 0.72\,(a/b) + 1.04$. The regression coefficient is significantly different from 1- ($t = -1.99$, $p<0.05$, d.f. = 140). The Y-intercept is not significantly different from 1- ($t = 0.40$, $p > 0.25$, d.f. = 140).

To test how well the data fit with the predictions of Model 2, regressions were carried out, taking $1/P_2$ as the dependent variable and $\frac{\log_e(k.a + e) - 1}{\log_e(k.b + e) - 1}$ as the independent variable. Different values of k were used in each regression. The results of the two regressions giving either a regression

TABLE 3.2. REGRESSION ANALYSIS TO EXAMINE THE RELATIONSHIP BETWEEN $1/P_2$ AND a/b FOR NR AND RN FEMALES

MODEL 1 ASSUMES COMPLETE SPERM MIXING AND LINEAR TRANSFER OF SPERM WITH TIME

The model predicts that:- $1/P_2 = a/b + 1$

where P_2 = proportion of eggs fertilized by second male

a = duration of mating with first male

b = duration of mating with second male

Regression analysis.

The regression equation is $1/P_2 = 0.721\ a/b + 1.044$.

Variable	b	S.E. b	t	d.f.	p	r.sq.
a/b	0.721	0.14	5.27	140	< 0.001	16.6%
(Y-intercept)	1.044	0.11	9.65	140	< 0.001	

coefficient or a Y-intercept value closest to those predicted from Model 2 are presented in Table 3.3. When *k* = 0.022, the regression coefficient is exactly equal to 1 and the regression coefficient does not differ significantly from 1 (Y-intercept = 0.814, t = -1.33, p > 0.2, d.f. = 140). When *k* = 0.002, the Y-intercept is exactly equal to 1 and the regression coefficient is not significantly different from 1- (b = 0.777, t = -1.48, p > 0.1). This regression also had the highest F-ratio of all the regressions taking *k* values from 0.001 to 1- (F = 27.81, p < 0.0005, d.f. = 140).

Durations of first and second matings

The mean duration of first matings (250 ± 43 minutes) was less than the mean duration of second matings (450 ± 72 minutes) These values represent the mean ± S.E. for 33 NN, RR, NR and RN females. A paired-sample t-test showed this to be a significant difference (t = -2.695, p < 0.02, d.f. = 32).

DISCUSSION

Changes in P_2 values over successive clutches

The fact that there was no statistically significant change in the proportion of eggs fertilized by second males over successive clutches indicates both that females can store sperm for considerable lengths of time without loss in

TABLE 3.3 REGRESSION ANALYSIS TO EXAMINE THE RELATIONSHIP BETWEEN $1/P_2$ AND $\frac{\log_e(k.a + e) - 1}{\log_e(k.b + e) - 1}$ FOR NR AND RN FEMALES

MODEL 2 ASSUMES COMPLETE SPERM MIXING AND DIMINISHING NUMBERS OF SPERM TRANSFERRED WITH TIME

The model predicts that $1/P_2 = \frac{\log_e(k.a + e) - 1}{\log_e(k.b + e) - 1} + 1$,

where P_2 = proportion of eggs fertilized by the second male,

a = duration of mating with first male,

b = duration of mating with second male,

k = a parameter.

REGRESSION ANALYSIS

i) $k = 0.022$

The regression equation is $1/P_2 = \frac{\log_e(0.022a + e) - 1}{\log_e(0.022b + e) - 1} + 0.814$

Variable	b	S.E. b	t	d.f.	p	r.sq.
$\frac{\log_e(0.022a + e) - 1}{\log_e(0.022b + e) - 1}$	1.000	0.19	5.19	140	< 0.001	16.1%
(Y-intercept)	0.814	0.14	5.62	140	< 0.001	

ii) $k = 0.002$

The regression equation is $1/P_2 = 0.777 \frac{\log_e(0.002a + e) - 1}{\log_e(0.002b + e) - 1} + 1.000$

Variable	b	S.E. b	t	d.f.	p	r.sq.
$\frac{\log_e(0.002a + e) - 1}{\log_e(0.002b + e) - 1}$	0.777	0.15	5.27	140	< 0.001	16.6%
(Y-intercept)	1.000	0.11	8.78	140	< 0.001	

fertility and that the mechanism of sperm competition operates only at the time of mating, or soon afterwards. This suggests that sperm precedence is not subsequently altered, for example, by any changes in the position of the sperm of different males in relation to the site of fertilization.

Similar results were obtained by Dickinson (1986, 1988) for the chrysomelid beetle, *L. c. clivicollis.* Females of this species were able to store sperm from a single mating of 15 hours for at least 30 days without a decline in the fertility of their eggs and there was no change in sperm precedence over 3 successive clutches laid by double-mated females.

Models to examine the mechanism of sperm competition

The results confirm that sperm competition does take place in *Gastrophysa viridula.* The data do not fit perfectly with the predictions of Model 1. Since the regression coefficient was significantly lower than predicted, there is some indication that the mechanism of sperm competition may be a combination of either a certain amount of sperm displacement or sperm repositioning with subsequent sperm mixing. The success of a particular male's sperm in fertilizing ova would then depend on the proportion of the total sperm stored by a female in a position to fertilize ova that his own represents after displacement or repositioning has taken place.

At first sight, this does not appear to be consistent with the findings of straight forward sperm mixing without displacement or repositioning in double matings reported by Shimizu and Fujiyama (1986) for the chrysomelid beetle, *Chrysolina aurichalcea*. However, the duration of each mating was relatively short (2 hours) in this case. Two such short matings may not have resulted in the females' sperm storage organs (spermathecae) being filled to capacity. If this were the case, sperm removal with longer mating durations remains a possibility in this species. This was what was found in the other chrysomelid beetle, *Labidomera c. clivicollis*, in which sperm competition has been investigated (Dickinson, 1986, 1988). When the total duration of double matings amounted to 30 hours there was no evidence for sperm removal. When the total duration of equal-length double matings was 90 hours, sperm of the second males predominated at fertilization. Together with the fact that there were no more sperm present in females' reproductive tracts after a double 45-hour mating than after a single 45-hour mating, these results indicate that sperm precedence of second males may result from time-dependent sperm removal and sperm mixing.

Walker (1980) found that the shape of the spermathecae in insects and mites may be related to the form of sperm competition that takes place in each species. He found that sperm displacement was commonly found in species with elongate or tubular spermathecae whereas spheroid or ovoid spermathecae were usually associated with little or no sperm displacement.

Dickinson (1986) reported that the spermatheca in *L. c. clivicollis* is U-shaped and sclerotized. Although her earlier work (Dickinson, 1986) was not in agreement with Walker's findings, her later work (Dickinson, 1988) is consistent with the trend for tubular spermathecae to be associated with sperm displacement. In *Gastrophysa viridula*, there is also a single, U-shaped, blind-ended tubular spermatheca. Sperm displacement in this species would be consistent with Walker's finding that sperm displacement is frequently associated with similarly-shaped spermathecae.

However, there is at present no direct evidence to confirm or refute the possibility of sperm removal in *G. viridula*. A pattern consistent with the hypotheseis of sperm displacement with sperm mixing could be caused by one of two mechanisms. Either sperm from previous males are repositioned within the spermatheca but nevertheless a certain amount of sperm mixing does take place. In common with other authors (Schlager, 1960; Riemann and Thorson, 1974; Brower, 1975), Walker (1980) suggested that in elongate spermathecae, sperm from previous males may more easily be displaced to a position where they cannot readily reach the spermathecal duct. Alternatively, as suggested for *L. c. clivicollis*, some previous sperm may be eliminated from the spermatheca, after which complete sperm mixing takes place.

Direct evidence for or against sperm from previous males being flushed out of females' reproductive tracts by later mating males could be obtained by labeling the first males' sperm and recording its presence after the females remate. All that can be said at present is that the data fitted to Model 1 are consistent with some form of sperm displacement along with sperm mixing in *G. viridula.*

An alternative interpretation of the possible mechanism of sperm competition is obtained when the data are fitted to Model 2. By choosing particular values of the parameter *k*, the data obtained fulfill the predictions of this model. This suggests that straightforward sperm mixing may occur, but that the amount of sperm transferred by males to females diminishes with time. The particular value of *k* that results in the best matching of the data to the predictions of the model is small (0.002). This represents a relationship between the number of sperm transferred and time that more closely resembles a linear relationship than if a larger value of *k* had been appropriate (see Figure 3.1).

From the present evidence it is difficult to draw any firm conclusions about the form that sperm competition takes in *Gastrophysa viridula.* A way of distinguishing between the two main alternative interpretations would be to count the number of sperm present in the reproductive tracts of females after matings of varying durations. This was done by Dickinson (1986) in the chrysomelid beetle, *Labidomera c. clivicollis.* She reported that there was a significant linear relationship between the number of sperm within females and the duration of mating. Such a finding in *G. viridula* would support the sperm displacement / mixing hypothesis. The fact that the spermatheca in this species is U-shaped and appears to be sclerotized and therefore inelastic also supports this hypothesis. If linear sperm transfer with time was found in *G. viridula*, it would then be useful to try to determine which form of sperm displacement occurs in this species. As mentioned earlier, sperm labeling could be used to determine the fate of first mating males after females have remated.

Durations of first and second matings

It was interesting to find that the mean duration of second matings was significantly greater than the mean duration of first matings. This may imply that males are able to recognize non-virgin females and alter their mating duration accordingly. Males could achieve a higher degree of paternity of eggs of a particular non-virgin female by mating for a longer time with her than previous males (Parker 1970b). The suggestion that males may be able to recognize different types of females is not new. Parker (1970b) found that matings with female dung flies, *Scatophaga stercoraria*, that had just laid their eggs were generally shorter than with females that contained at least some mature eggs. This suggested that the males were able to distinguish between these two types of females.

However, if there is a selective advantage to later mating males in extending their mating times, it would also be likely that males mating with virgins would be selected to have long mating times to reduce their loss of paternity due to females remating with other males. Dickinson (1988) suggested that male *L. c. clivicollis* remain with females until the females are ready to lay eggs both in order to increase the extent to which they supercede the sperm of previous males and to minimize the likelihood that the females will remate again before laying her next clutch of eggs. This could lead to an overall pattern of lengthy matings with both virgin and non-virgin females.

It is difficult to attach a great deal of significance to the finding that second matings were generally lengthier than first matings. The males of *Gastrophysa viridula*, although forming fairly long mating associations with particular females, appear to mate intermittently with them. This also seems to be the case in *Labidomera c. clivicollis* in which mating associations lasted for up to 2.5 days in the field (Dickinson, 1986,1988). In the present experiment, each male was confined in a small petri dish with only one female. Under these conditions it was difficult to determine the point at which a mating association would "naturally" have ended. An arbitrary rule was chosen such that if a male did not remount the female within half an hour of last dismounting, the mating was considered to be over. But it is important to recognize that this was only an arbitrary rule and may not reflect what would happen in a natural situation.

Whatever the precise mechanism of sperm competition in *Gastrophysa viridula*, it appears that the eggs within a particular clutch may be a mixture of full- and half-siblings. Without field data it is not possible to be more specific than that. The more males a female mates with, the smaller the proportion of full-siblings she is likely to have in her clutches. On the other hand, if the finding that later matings with a female are generally longer than earlier ones holds true under natural circumstances, the proportion of full-siblings in clutches is likely to be increased.

There are always likely to be at least some full-siblings within a clutch. The degree of relatedness between the eggs of a particular clutch will therefore lie somewhere between 0.25 and 0.5. This implies that, unless larvae are able to distinguish full-siblings from half-siblings, within-clutch cannibalism or cannibalism of eggs from other clutches laid by a larva's own mother will be selected for only if the ratio of fitness gained by a cannibal to fitness lost by a victim is greater than somewhere between a half and a quarter. The ability of larvae to distinguish between kin and non-kin will be examined in Chapter 6.

CHAPTER 4

THE RELATIONSHIP BETWEEN THE AGE OF *G. VIRIDULA* EGGS AND THEIR SUSCEPTIBILITY TO CANNIBALISM

Outline of Chapter 4

In this chapter the relationship between the age of *Gastrophysa viridula* eggs and their susceptibility to cannibalism by first instar larvae is examined. This is followed by a discussion of the possible physical and chemical properties of the eggs that might influence this relationship. A preliminary investigation of one of these chemical properties is presented and an outline of planned future research in this area is given.

4.1. THE RELATIONSHIP BETWEEN THE AGE OF *G. VIRIDULA* EGGS AND THEIR SUSCEPTIBILITY TO CANNIBALISM

INTRODUCTION

As shown in Chapter 2, the extent of within-clutch cannibalism can be influenced by the number of eggs that females lay in their clutches. With smaller clutch sizes there is a greater degree of synchrony of hatching and viable eggs may escape being cannibalized by temporal means - larvae cannot become cannibalistic until they have left their egg shells, by which time a large proportion of the viable eggs in small clutches will already have hatched.

This means of protection is not perfect. Some viable eggs remain unhatched when the first of the larvae have already left their egg shells.

These eggs are then vulnerable to cannibalism and a small number of them are sometimes attacked (see Chapter 2). But even so, some still manage to hatch successfully. It was not clear whether the eggs that escaped being cannibalized did so because they were not encountered by cannibalistic larvae, or because they were in some other way protected against such predation.

An experiment was designed to test the hypothesis that eggs close to the hatching stage are protected against cannibalism by means other than temporal avoidance. The extent of cannibalism inflicted by first instar larvae on eggs of different ages, from the day of laying through to the day of hatching, was examined.

METHODS

Female beetles of similar ages were kept individually in petri dishes lined with moistened filter paper and supplied with a piece of dock leaf. The laboratory conditions were maintained at 20° ± 1° C. with a 16h:8h light:dark photoperiod. On the mornings when collection of eggs was to be carried out, the petri dishes were emptied of any eggs that had been laid during the night and the dock leaves were replaced by fresh pieces. Newly laid eggs were then obtained by checking the petri dishes every half hour. On discovery of a new clutch, two lots of 16 eggs were taken from it using a moist paintbrush.

One lot was placed in a small, 5-cm. diameter petri dish labelled 'A'. The second lot was placed in a similar petri dish labelled 'B'. Both petri dishes were lined with moistened filter paper and dock leaf cut to the size of the container with a pastry-cutter. The eggs in all 'A' petri dishes developed through to hatching in the laboratory. The corresponding 'B' eggs developed for varying periods of time (0, 24, 48, 72, 96 or 108 hours) under the same conditions. They were then transferred to a cold storage room with conditions of $4^{\circ} \pm 1^{\circ}$ C. and 12h:12h light:dark photoperiod to arrest their development.

When hatching of the 'A' eggs had reached the stage where all larvae had their head and thorax protruding from the egg shell, the corresponding 'B' eggs were placed in a circle immediately around the larvae. In this way, newly hatched larvae were presented with eggs of different "ages". Since not all 16 'A' eggs hatched in every lot, the number of larvae that did hatch was noted.

After 24 hours the 'B' eggs were examined under a binocular microscope and the eggs eaten by the 'A' larvae were scored as shown on the following page:-

Proportion of each egg eaten (x)	Score
Not eaten	0
$0 < x \leq 0.25$	0.125
$0.25 < x \leq 0.5$	0.375
$0.5 < x \leq 0.75$	0.625
$0.75 < x \leq 1$	0.875

A total score was obtained for each group of 16 'B' eggs. All counting and manipulation of eggs was done under sterile conditions in a laminar flow cabinet to minimize the risk of fungal infection on the eggs.

Stepwise multiple regression was used to examine the effects of the age of the eggs and the number of larvae that hatched out on the score of eggs eaten and on the number of eggs at least partially eaten in each trial.

The effect of the cold storage room conditions on the development of the eggs was tested during an experiment on kin recognition (see Chapter 6). The results showed that the cold room conditions did not completely halt the development of eggs. The 'B' eggs presented to the 'A' larvae would therefore have been of a more advanced developmental age than the number of hours that they had spent in the laboratory. However, this time does give a rough approximation of "age" for the purposes of this experiment.

The effect on the viability of eggs placed in the cold storage room was also tested during the experiment on kin recognition. The viability of eggs was not affected by being stored in these colder conditions.

RESULTS

In Figure 4.1.1. the score of eggs eaten is plotted against the age of the eggs and the number of larvae that hatched. Stepwise multiple regression showed that both the number of larvae that hatched out from the 'A' groups of eggs and the age of the eggs did have an effect on the score of eggs eaten (Score of eggs eaten = -0.09 egg age + 0.67 no. of larvae + 1.43. F=76.53, $p<0.0001$, r. sq.=64.84%, d.f.=2,83). Not surprisingly, the greater the number of larvae, the higher the score of eggs eaten. With increasing age of the eggs, there was a significant decrease in the score of eggs eaten (see Figure 4.1.1.).

This effect was not merely due to smaller proportions of each older egg being eaten. Stepwise multiple regression showed that the greater the number of larvae, the greater the number of eggs at least partially eaten in trials and that with increasing age of the eggs there was a significant decrease in the number of eggs at least partially eaten (Number of egs at least partially eaten = -0.12 egg age + 0.85 no. of larvae + 1.87. F=72.14, $p<0.0001$, r.sq.=63.48%, d.f.=2,83). The mean number of 0-hour eggs partially eaten in trials with 16, 15, 14 and 13 larvae was 14.6 ± 0.5 (mean ± S.E. for 10 trials). The mean number of 108-hour eggs at least partially eaten in trials with 16

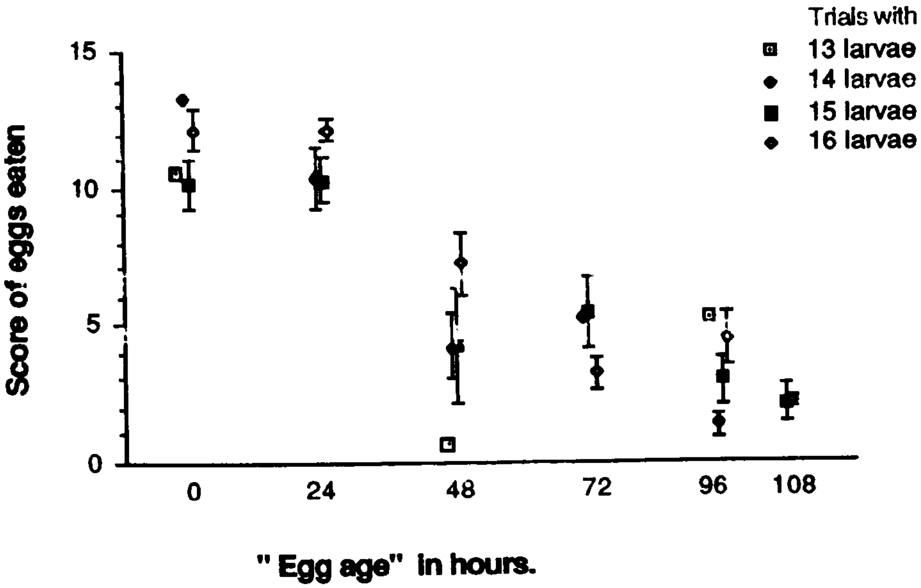
FIGURE 4.1.1. **THE RELATIONSHIP BETWEEN SCORE OF EGGS EATEN AND EGG AGE**

Each point represents the mean ± one standard error (except where only one trial was obtained with eggs of a certain age and a particular number of larvae).

STEPWISE MULTIPLE REGRESSION

The regression equation is Score of eggs eaten = -0.09 egg age + 0.67 no. of larvae + 1.43. (F = 76.53, $p < 0.0001$, r.sq. = 64.84%, d.f. =2,83)

Variable	b	S.E.	t	p
Egg age	-0.09	0.01	-12.37	< 0.0001
No. of larvae	0.67	0.32	0.14	0.043
(Constant)	1.43	4.94		


Trials with
13 larvae
14 larvae
15 larvae
16 larvae
Score of eggs eaten
15
10
5
0
0
24
48
72
96
108
" Egg age" in hours.

and 15 larvae was 2.5 ± 0.42 (mean ± S.E. for 16 trials) - no trials were obtained for 108-hour eggs with 14 or 13 larvae. All but 2 of the 216 untouched 108-hour eggs subsequently hatched out successfully.

DISCUSSION

Age - dependent protective mechanism of eggs in G. viridula

This experiment has established that the eggs of *Gastrophysa viridula* do have a mechanism of protection against within-clutch cannibalism that is independent of temporal avoidance through synchrony of hatching. This additional mechanism does not provide absolute protection for all viable eggs within a clutch. This was demonstrated in Chapter 2, where it was shown that some viable eggs are eaten from within clutches at hatching.

In this experiment the possibility that the 108-hour eggs that were at least partially eaten represent only non-viable eggs seems highly unlikely. A mean of $13.6 \pm^{2.9}_{2.7}$% ($n = 16$ trials) of 108-hour eggs were at least partially eaten. The percentage of non-viable eggs normally found in clutches is much lower than that (see Chapter 2). In the artificially reduced 'A' clutches of this experiment, the mean frequency of non-hatching was $1.9 \pm^{0.5}_{0.5}$% ($n = 86$ trials). It seems possible that this intrinsic mechanism of protection may be effective in reducing the extent of cannnibalism of viable eggs within a clutch to a level below that already achieved through relatively synchronous hatching. The avoidance of cannibalism by some unhatched viable eggs observed in clutches when hatched larvae have already begun to eat eggs may be at least partly attributable to this protective mechanism.

The extent of cannibalism was reduced even in 48-hour eggs. This suggests that the eggs may be protected to some degree not only against within-clutch cannibalism, but also against cannibalism at an earlier stage from larvae from other clutches. Inter-clutch cannibalism will be examined more closely in Chapter 6. The extent of cannibalism of 0-hour and 24-hour eggs was very similar and so eggs at this early stage of development are probably not intrinsically protected to a very great degree. However, the possibility that females may lay their eggs in such a positon as to avoid inter-clutch cannibalism from at least first instar larvae is also discussed in Chapter 6.

Depending on the nature of the protective mechanism discovered in this experiment, the older eggs of *Gastrophysa viridula* may also be more protected against predation from other species than younger eggs. This remains an idea to be tested in the future.

Age - dependent protective mechanism of eggs in other species

The resistance of older eggs to cannibalism has been shown in other species of invertebrates. Canard (1970) has shown that older eggs of the aphidophagous chrysopid species, *Chrysopa perla*, are protected against cannibalism. Unfortunately, it is not known at what stage this protective mechanism becomes effective. Eggs from 0-24 hours of age were always consumed when presented to first instar larvae. Eighty percent of 5-7 day old eggs were rejected, but 90% of eggs were cannibalized on the eighth day when they were due to hatch.

It is interesting to note that the degree of intrinsic protection of *Chrysopa perla* eggs is considerably reduced on the day of hatching. *Chrysopa perla* eggs are not laid in compact clutches as are *Gastrophysa* eggs. Although Canard found that an average of 18.5 eggs are laid in one lot, each egg is laid in isolation from other eggs. The chances of larvae encountering eggs from within their own lot are low. But even if they did, these eggs are additionally protected by having been laid on a pedicel. Canard showed that until the larvae are at least half a day old, they will not undertake any exploration of a pedicel to reach an egg. From the point of view of within-clutch cannibalism at least, the need for intrinsic protection of the eggs will not be so critical on the day of hatching for *Chrysopa perla* as it is for *Gastrophysa viridula*. It would be interesting to know at what stage the intrinsic protection of *Chrysopa perla* eggs becomes effective and how useful this is in terms of protecting against inter-clutch cannibalism and predation from other species.

The springtail *Sinella curviseta* is another example of a species in which the older eggs are protected against cannibalism. Waldorf (1971) presented the results of an experiment in which adult springtails could choose to eat either smooth, spherical eggs less than 24 hours of age or rough, slightly elongate eggs greater than 24 hours of age. Almost all the eggs consumed (99%) were of the smooth type. Egg consumption occurs at a significantly higher rate in females than in males, and females are most likely to encounter their own eggs in nature. The advantages of an early protection mechanism for the eggs can be clearly seen. It would be detrimental to the inclusive fitness of females to eat their own viable offspring and it would certainly be detrimental from the eggs' point of view! Waldorf (1971) stated that for the majority of time in which adults are exposed to a clutch of eggs, all of the smooth eggs will be faulty. She concluded that it represents an efficient use of resources to consume eggs that have a hatching success probability of zero.

This does not seem to be the case for *Gastrophysa viridula*. The results presented in Chapter 2 indicate that viable eggs are sometimes eaten from within a clutch as well as non-viable eggs.

The selective benefits of an anti-cannibalistic protective mechanism

The selective benefits of a protective mechanism against cannibalism are not difficult to see. In the case of within-clutch cannibalism, any eggs having this would be less likely to be eaten than unprotected eggs. From the females' point of view, those laying eggs with intrinsic protection could probably achieve a higher lifetime production of successfully hatched larvae than those laying eggs without this protection. A female may be able to gather a fixed amount of food resources to mature a certain number of eggs over her lifetime. A female that lays eggs unprotected from intra-clutch cannibalism may be forced to invest more resources per surviving offspring, and so produce fewer of them, than females that lay clutches protected from this form of cannibalism. This would be true providing that any costs to females investing resources in the protection of eggs did not result in their producing fewer eggs than the number of surviving larvae produced by females laying unprotected eggs.

The possibility that larvae may gain individual benefits from eating viable eggs from within clutches should also be considered. If this were the case, females laying eggs without intrinsic protection would produce fewer, but fitter, offspring than other females. The females' best strategy would then depend on whether or not the benefits gained by her cannibalistic offspring were sufficient to outweigh the loss of a certain number of her viable eggs. The possible individual benefits of cannibalism were investigated and are discussed in the next chapter.

It was found that cannibals gain benefits over non-cannibals in terms of their developmental rates and survival. However, there was some evidence to suggest that eating older, viable eggs may not be beneficial and may in fact be detrimental. This requires more direct confirmation. If it is the case, however, the intrinsic protection mechanism of eggs against cannibalism can be seen as being selectively advantageous, not only to the eggs themselves, but also to the mothers that laid them.

This would also be true for the case of inter-clutch cannibalism. The sacrifice of an individual could never be repaid, in genetic terms, if it was eaten by an unrelated individual. Likewise, the inclusive fitness of a female would be decreased if her eggs were eaten by unrelated larvae. There can therefore be little doubt about the selective advantages of an intrinsic protective mechanism against cannibalism for both eggs and females.

The possible nature of the mechanism protecting older eggs from cannibalism will be discussed in the next section. This leads to some speculation as to why this intrinsic protective mechanism is not sufficient to prevent cannibalism completely.

4.2. THE POSSIBLE NATURE OF THE INTRINSIC PROTECTIVE MECHANISM OF *G. VIRIDULA* EGGS

INTRODUCTION

There are at least two ways in which the eggs of *Gastrophysa viridula* could become increasingly immune to cannibalism with age. The first possibility is that as the eggs grow older, they become mechanically more resistant to the piercing mouthparts of the larvae. This idea was tested by Canard (1970) for the eggs of *Chrysopa perla.* He used tactile needles to judge the force required to rupture the embryonic membranes of eggs of different ages.

The force required did not increase greatly during the first two days of incubation and single eggs of this age were always consumed when presented to first instar larvae. During the third and beginning of the fourth days there was a dramatic increase in the mechanical resistance of the eggs, stabilizing between the fifth and seventh days at a level seventeen times greater than that of newly laid eggs. Only 20% of eggs between 5 and 7 days old were consumed when presented to first instar larvae. During the eighth day of incubation the force required to rupture the embryonic membranes declined to a little less than seven times the initial value. Correspondingly, 90% of eggs on this last day of incubation were eaten when presented to first instar larvae. Canard concluded that first instar larvae were prevented from eating older eggs until the last day of incubation by the mechanical resistance of the embryonic membranes.

The second possibility is that chemical defence substances may develop with age in the eggs. Several authors have noted that chrysomelids often form dense, conspicuous colonies on their food plants and are therefore highly exposed to predators and parasitoids (e.g. Force, 1966; Daloze & Pasteels, 1979; Howard *et al.*, 1982; Pasteels *et al.*, 1982, 1984, 1986; Daloze *et al.*, 1986). These authors suggest that the various defence mechanisms that are found in the family have evolved as a means of protection against such predation and parasitism.

The defence mechanisms of chrysomelids include mechanical defence, reflex bleeding and feigning death in adults and chemical defence in all stages of their life history (Pasteels *et al.*, 1982). Chemical defence secretions were originally studied mostly in the adult and larval stages of leaf beetles. But since the eggs of many chrysomelids are bright orange or yellow in colour and are laid in clusters on the foliage of the host plant, they too will be conspicuous and vulnerable to predation. This has led to the inclusion of the egg stage in more recent investigations of chrysomelid chemical defence mechanisms (Howard *et al.*, 1982; Pasteels *et al.*, 1986).

Two different types of defensive chemicals have been found in the eggs of species belonging to the subfamilies Chrysomelina (of which *Gastrophysa viridula* is a member), and Phyllodectina. Isoxazolinone glucosides are one of the types of compounds found (Pasteels *et al.*, 1986). One of these contains a nitropropionate group. It was found to be highly deterrent at natural concentrations to the feeding behaviour of a potential egg predator, the ant *Myrmica rubra.* The other compound does not contain a nitropropionate. It was found to be neither highly toxic nor deterrent to ants and is thought to be simply an inactive precursor of the nitropropionate-containing compound (Pasteels *et al.*, 1986).

The other major compounds found in chrysomelid eggs that are of interest from a chemical defence point of view are lipids. Attention has been focused on oleic acid in particular. It has been found in the eggs of *Gastrophysa viridula* (Pasteels *et al.*, 1986), and the American *Gastrophysa cyanea* (Howard *et al.*, 1982). In *Gastrophysa cyanea* the eggs were found to contain quite substantial quantities of oleic acid (40µ g/egg). Howard *et al.* found that at this concentration, oleic acid was an effective feeding deterrent for several species of ants. In fact, the deterrent properties of oleic acid have been demonstrated several times in experiments where objects coated in it have been rejected from the nests of various species of ants (Wilson *et al.*, 1958; Blum, 1970; Haskins, 1970; Howard & Tschinkel, 1976). In contrast to the situation in *G. cyanea*, Pasteels *et al.* (1986) found that only trace amounts of oleic acid were present in the eggs of *Gastrophysa viridula*

(0.065μg/egg). They concluded that oleic acid is unlikely to be involved in the defence of the eggs.

However, it is not clear whether it is free oleic acid *per se* that is important in chemical defence or whether oleic acid incorporated in other lipid compounds could be equally effective in deterring predators. Iltis and Zweig (1962) found that the apical drop on the posterior pole of eggs of some culicine mosquitoes contains glycerides of several fatty acids. Hinton (1968) subsequently discovered that the apical drop material from the mosquito *Culex pipiens* interfered with predation by ants when it was used to coat otherwise acceptable prey items. This provides evidence that fatty acids may be effective deterrents in glyceride form. However, this possibility seems to have been ignored in studies investigating the role of fatty acids in chemical defence. Pasteels *et al.* (1986) did mention that the major lipids present in *G. viridula* eggs were triglycerides. But they made no comment as to the possible significance of this finding in terms of chemical defence.

In discussing the defensive chemistry of chrysomelid eggs, it has so far always been assumed that such defences have evolved as a means of protection against interspecific predation (Howard *et al.*, 1982; Pasteels *et al.*, 1986). However, cannibalism is potentially an important form of predation, at least in the green dock leaf beetle. It would be interesting to consider the possibility that chemical defences may also play a part in the protection of eggs against predation from within their own species.

Howard *et al.* (1982) and Pasteels *et al.* (1986) did not state the age of the eggs that they used to test for the presence of oleic acid and isoxazolinone glucosides. This would be an important piece of information to have when considering any possible links between chemical defences and protection against cannibalism. As seen in Section 1 of this chapter, the susceptibility of eggs to cannibalism decreases with the age of the eggs. If the concentration of any of the defensive chemicals in the eggs were found to increase with age in a corresponding manner, suggestive evidence would be provided that these substances are implicated in the defence of eggs against cannibalism.

As a preliminary investigation, the relative concentrations of the major fatty acids (with free and incorporated forms taken together) were examined in eggs of different ages, from the day of laying through to the day of hatching.

METHODS

Before starting this experiment, the hatching success rate in clutches of eggs laid by adult females of similar ages was checked. This was done to ensure that none of the females were laying clutches with abnormally high numbers of non-viable eggs.

On each of six consecutive days, the females were isolated between the hours of 10 a.m. and 8 p.m. in 9 cm. petri dishes. The dishes were lined with filter paper moistened with distilled water and provided with a fresh piece of dock leaf. At the end of this period each day, three samples of 200 eggs were collected. Each sample was carefully placed in a small petri dish lined with moistened filter paper and stored under the same temperature and photoperiod conditions as the adult beetles - 20°± 1°C and 16h:8h light:dark photoperiod. All handling of eggs and adults was carried out under sterile conditions in a laminar flow cabinet to minimize the risk of fungal infections on the eggs.

Extraction of lipids from the egg samples was begun immediately after the egg samples for the sixth day had been collected. Only one sample of 200 eggs was eventually obtained from the first day of collection. On the sixth day, many of the first-day eggs had hatched out before extraction was begun, leaving only enough to make up one sample of 200 eggs.

Each sample of eggs was ground using a mortar and pestle with acid washed sand and transferred to a cellulose extraction thimble. The mortar was cleaned with a further portion of sand and this material was also transferred to the thimble. The mortar and pestle were wiped with cotton wool soaked in dichloromethane (CH_2Cl_2) which was then used to stopper the thimble. All sixteen samples took just under two hours to process in this way and this time was taken into account when estimating the "age" of the eggs. The ground samples were stored over-night in a freezer.

The following day, each sample was extracted by continuous extraction in Butt (Bolton) extractors using CH_2Cl_2 for two hours. The thimble was removed and the sample reground and extracted for one hour more. The solvent was evaporated off under vacuum and methyl esters were prepared for Gas Liquid Chromatography (G.L.C.).

5 ml. alcoholic NaOH (20g./l.) was added to each sample extract for 30 minutes. The purpose of this alkaline hydrolysis step was to yield fatty acid salts from lipids in the extract that contained fatty acids as components.

5 ml. boron trifluoride-methanol complex was then added to each extract. At this stage fatty acid methyl esters were formed.

1 ml. heptane was added and mixed. Saturated NaCl solution was used to raise the level of heptane to the neck of the flask and a portion of the heptane solution of fatty acid methyl esters was then dried over sodium sulphate. These samples were kept refrigerated until the G.L.C. was run.

For G.L.C. analysis, 1 μl. of the heptane solution of fatty acid methyl esters from each sample was injected onto a 25 m. x 0.3 mm. i.d. O.V. 351

(FFAP) WCOT with split injector at 210 isothermal using N_2 as the carrier gas. Peaks were identified by comparison with known standard fatty acid methyl ester mixtures. The relative concentration of each fatty acid was calculated as a percentage of the total fatty acid concentration in each sample.

Regression analysis was used to examine the relationships between the relative concentrations of the major fatty acids present and the age of the eggs. The percentages were arcsine transformed for the analysis.

RESULTS

The five major fatty acids present in the egg lipid extracts were identified as being palmitic acid (16:0), stearic acid (18:0), oleic acid ($18{:}1\Delta^{9}$), linoleic acid ($18{:}2\Delta^{9,12}$), and linolenic acid ($18{:}3\Delta^{9,12,15}$).[*] Other fatty acids were present, but only in relatively very minor proportions and they were not present in all of the samples. They were not, therefore, included in the following analysis.

---

[*] Fatty acids are symbolized by a shorthand notation. The first number designates the length of the carbon chain, the second number designates the number of double bonds and the position of the double bonds are represented by the triangle symbol followed by superscript numbers. It is understood that the double bonds are *cis* unless indicated otherwise.

Regression analysis showed that the relative proportions of palmitic, linoleic and stearic acids did not significantly change with egg age (See Figure 4.2.1.b). However, the relative proportion of oleic acid significantly increased with the increasing age of the eggs; that of linolenic acid significantly decreased (Figure 4.2.1a).

DISCUSSION

This experiment was carried out simply by way of a very preliminary investigation. Rather than providing answers to any important questions, the results serve only as an indication that it would be worthwhile pursuing this line of enquiry. Further experiments were planned, but after the completion of this initial experiment, difficulties were encountered in maintaining the laboratory stocks of beetles. At the same time, similar problems were being experienced with the stocks of beetles held at Lancaster University. Since beetles in the field had already entered diapause, no source of beetles was available to work with; so the experiments could not be carried out.

The possible involvement of oleic acid in the protection of eggs against cannibalism

As oleic acid was the only fatty acid to increase in relative concentration with the age of the eggs, its possible protective role would certainly be worth looking at in greater depth. First of all, the part played by oleic acid in the

FIGURE 4.2.1. **THE RELATIONSHIPS BETWEEN THE RELATIVE CONCENTRATIONS OF THE MAJOR FATTY ACIDS PRESENT IN *G. VIRIDULA* EGGS AND THE AGE OF THE EGGS**

Each point represents the relative concentration of a fatty acid in 200 eggs (as arcsine % of total major F.A. concentration)

REGRESSION ANALYSIS

a) The relationship between the relative concentration of F.A. and egg age was significant in the cases of oleic and linolenic acid.

OLEIC ACID - relative conc. oleic acid = 30.9 + 0.08 egg age.

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	129.39	24.46	< 0.0005	63.6%
Error	14	5.29			
Total	15				

LINOLENIC ACID - relative conc. linolenic acid = 29.5 - 0.12 egg age.

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	312.24	22.63	< 0.0005	61.8%
Error	14	13.79			
Total	15				

b) The relationship between the relative concentration of F.A. and egg age was not significant in the case of palmitic, stearic and linoleic acid.

PALMITIC ACID - relative conc. palmitic acid = 32.0 + 0.02 egg age.

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	12.84	1.31	> 0.25	8.6%
Error	14	9.78			
Total	15				

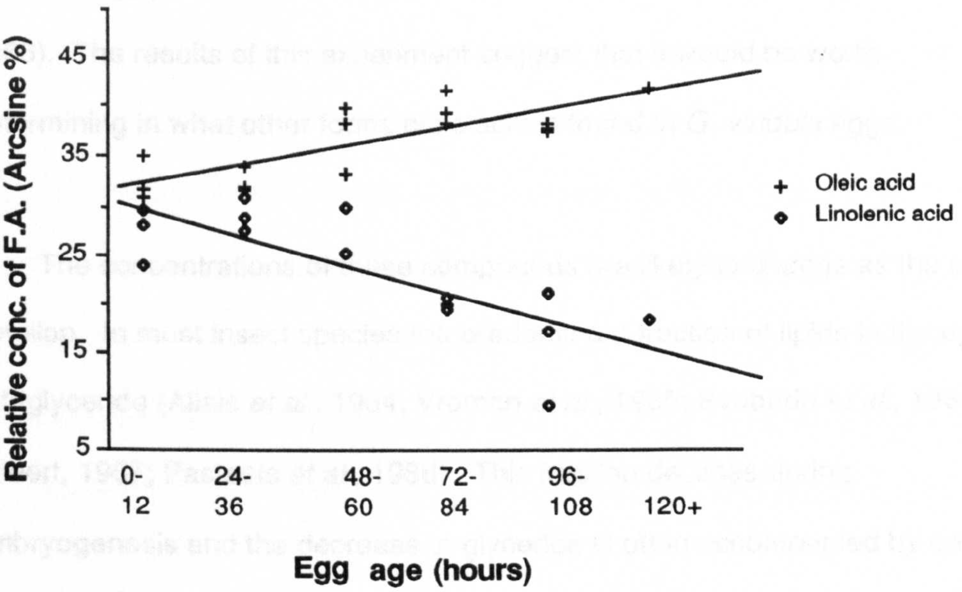
STEARIC ACID - relative conc. stearic acid = 18.1 - 0.003 egg age.

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	0.23	0.27	> 0.25	1.9%
Error	14	0.84			
Total	15				

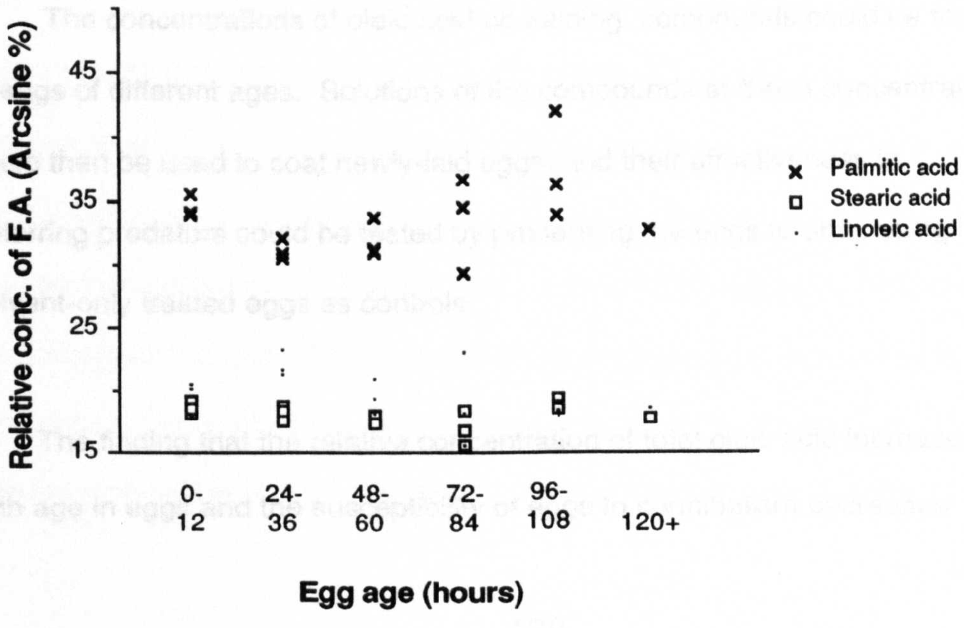
LINOLEIC ACID - relative conc. linoleic acid = 20.7 - 0.02 egg age.

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	12.25	2.81	> 0.1	16.7%
Error	14	4.36			
Total	15				

a)


Relative conc. of F.A. (Arcsine %)
45
35
25
15
5
0-
12
24-
36
48-
60
72-
84
96-
108
120+
Egg age (hours)
+ Oleic acid
◇ Linolenic acid

b)


Relative conc. of F.A. (Arcsine %)
45
35
25
15
0-
12
24-
36
48-
60
72-
84
96-
108
120+
Egg age (hours)
× Palmitic acid
□ Stearic acid
· Linoleic acid

defence of eggs against predation from other species could be examined further. So far, only free oleic acid has been shown to be effective in deterring potential egg predators such as ants (Howard *et al.*, 1982; Pasteels *et al.*, 1986). The results of this experiment suggest that it would be worth determining in what other forms oleic acid is found in *G. viridula* eggs.

The concentrations of these compounds are likely to change as the eggs develop. In most insect species the predominant fraction of lipids in the eggs is triglyceride (Allais *et al.*, 1964; Vroman *et al.*, 1965; Svoboda *et al.*, 1966; Gilbert, 1967; Pasteels *et al.*, 1986). This fraction declines during embryogenesis and the decrease in glyceride is often accompanied by an increase in phospholipid (Niemerko *et al.*, 1956; Gilbert, 1967; Yurkiewicz & Oelsner, 1969; Lipsitz & McFarlane, 1970, 1971). It would be interesting to find out which of these lipids, if any, affect the protection of the eggs.

The concentrations of oleic acid-containing compounds could be found in eggs of different ages. Solutions of the compounds at these concentrations could then be used to coat newly-laid eggs, and their effectiveness in deterring predators could be tested by presenting the eggs to ants, using solvent-only treated eggs as controls.

The finding that the relative concentration of total oleic acid increases with age in eggs and the susceptibility of eggs to cannibalism decreases suggests a possible link between these two observations. Oleic acid and oleic acid-containing compounds might be effective anti-cannibalistic agents. This possibility could be tested in a similar way to that described for testing their effectiveness against predation. In this case, the predators used would be newly hatched larvae instead of ants. The absolute concentration of free oleic acid could be found by omitting the alkaline hydrolysis step described in the methods for this section and using a sample of methyl oleate as a standard for quantitative G.L.C. analysis. Diazomethane (CH_2N_2) might be preferable as the methylating reagent when analyzing the content of free oleic acid because of the mildness of its reaction conditions (Black,1983).

The possible involvement of other defensive chemicals in the protection of eggs against cannibalism

The possibility that one of the isoxazolinone glucosides might be involved in the protection of eggs against cannibalism could be tested in a similar way to that described for oleic acid-containing compounds. The concentration of the isoxazolinone compounds in eggs of different ages could be determined by firstly extracting with chloroform : methanol (1:1 v/v) and then performing quantitative analysis by photodensitometry using thin-layer chromatography plates. The pure isoxazolinone glucosides to be used as standards could be obtained by performing column chromatography on the defensive secretion of adult beetles. Clusters of defence glands open into grooves on the surface of the pronotum and the elytra (Deroe & Pasteels, 1982). When the beetles are disturbed the secretion oozes from the openings of the glands, covers the integument and accumulates in the marginal grooves where it can easily be collected on small pieces of filter paper (Pasteels *et al.*, 1984). The compounds could once again be used to coat newly-laid eggs in the same concentrations as found in eggs of different ages. Solvent-only treated eggs would be used as controls.

The possibility that mechanical defence may play a part in protecting older eggs of *Gastrophysa viridula* in a similar way to that suggested by Canard (1970) has not yet been investigated. However, the fact that some older, viable eggs are at least partially eaten within clutches is suggestive that chemical defence may be involved in the intrinsic protection of *G.viridula* eggs. The two isoxazolinone glucosides and oleic acid have been found in the fluid contents of the eggs of *Gastrophysa viridula*, but not in the gluey secretion covering the eggs (Pasteels *et al.*, 1986). The position of these chemicals means that they could only be effective in deterring predators or cannibals once an egg had been punctured and some of the fluid contents sucked out.

Such a defence mechanism could therefore never completely prevent cannibalism or predation from other species. It could, however, prevent total loss of a clutch of eggs. This type of defence against predation was first considered by Fisher (1930). He discussed the selective benefit of the sacrifice of one victim in order to save possibly numerous siblings within the same brood or clutch. Hamilton (1964 b) greatly extended this idea, noting that further selective benefit could occur through saving more distant relatives.

That this form of defence is effective against inter-specific predation for *Gastrophysa viridula* eggs has already been established (Pasteels *et al.*, 1986). The interesting question is whether or not the same mechanism works against cannibalism. If this were the case, it is worth noting that in the 108-hour eggs, only a very few were at all damaged. The majority of the eggs were not attacked. If chemical defence is involved, this would imply that the destruction of a very small number of eggs can protect the majority within a clutch, even when being attacked by as many as 16 larvae.

CHAPTER 5

THE NUTRITIONAL BENEFITS OF CANNIBALISM IN *G. VIRIDULA*

Outline of Chapter 5

In this chapter the possible individual fitness benefits to be gained from eating conspecific eggs are investigated. The adequacy of eating eggs alone in order to complete larval development is first of all examined. The effects of adding conspecific eggs to a diet of dock leaves on the survival, developmental rates and attained adult weights of larvae are also explored. The relationships between adult female size and longevity and measures of fecundity are considered, and the discovery of an interesting pattern in the egg numbers laid by females in successive clutches is discussed.

INTRODUCTION

Gastrophysa viridula larvae, given the opportunity, will readily eat eggs both from within their own clutches and from other, unrelated clutches. This suggests that cannibalism may be advantageous in terms of their own individual fitness. There are several ways in which individuals might benefit from cannibalistic behaviour. By eating conspecifics, they may reduce competition for limited resources such as territory or food (Fox, 1975; Polis, 1981).

Larvae of the alfalfa blotch leafminer, *Agromyza frontella* (Rondani) complete their development within the alfalfa leaflet in which the eggs are laid and are often limited by food resources when there is more than one larva per leaflet. In laboratory studies, Quiring and M^{c}Neil (1985) found that in cases where there were originally two larvae per leaflet, cannibals that eliminated the other larva had higher pupal weights and overall survival than non-cannibals developing two per leaflet. They also found that cannibals completed their development slightly faster than individuals that had developed alone in the leaflet from the start. However, the pupal development time and the fecundity of mated females did not differ significantly between cannibals and non-cannibals. They therefore concluded that the most significant effect of cannibalism in this case was to reduce exploitation competition by eliminating a potential competitor.

In *Gastrophysa viridula*, it seems unlikely that the elimination of conspecific competitors would be the most important factor favouring cannibalistic behaviour. Larvae are not confined to a food source of strictly limited carrying capacity for their development. They are free to move between the leaves of an individual dock plant and also between neighbouring plants. Except at extremely high population densities, competition for food will not normally be a problem. Eickwort (1971) also reported that there was little or no competition for food among larvae of a milkweed beetle, *Labidomera clivicollis*, which is another clutch laying, cannibalistic chrysomelid beetle.

By eating their conspecifics, cannibals are obtaining an additional source of food. If these conspecifics were in some way nutritionally superior to, or contained important nutrients lacking in other items of an animal's diet, cannibals would be at a selective advantage over non-cannibals and cannibalism would be expected to evolve. Hoogland (1985) suggested that infanticide and cannibalism of closely related individuals in prairie dogs, *Cynomys ludovicianus*, provided nutritional benefits to lactating females. Meffe and Crump (1987) found that when conspecifics were added to a basic commercial food diet given to mosquitofish, *Gambusia affinis*, the fish showed improved measures of somatic growth and reproduction. Other supplements of items normally found in the natural diet of mosquitofish did not have this effect. A trend, though not significant, was also found toward larger brood sizes being produced by the cannibals than the fish fed on the other diets. Nagai *et al.* (1971) found that tadpoles of the Japanese toad, *Bufo vulgaris*, which fed on conspecifics showed a more efficient conversion rate of amino acids than tadpoles fed on an artificial food or fish-meal diet.

Invertebrates too have been found to gain nutritional benefits from cannibalism. Baur (1986) found that newly hatched land snails, *Arianta arbustorum*, fed with eggs of the same species over a period of two weeks produced an increase in shell diameter corresponding to a 500% increase in weight. Snails fed on lettuce over the same period produced an increase in shell diameter corresponding to a weight increase of only 50%. Baur attributed this difference in weight gain to the fact that *A. arbustorum* eggs have a much higher protein content than lettuce.

In insects, cannibalism has been associated with increases in survival, fecundity and developmental rate. In several species of aphidophagous coccinellids, an increase in the period of survival of cannibalistic first instar larvae as compared with the survival of unfed larvae has been reported. Coccinellid larvae are unable to move rapidly in search of food if aphids are not present where they hatch out and they cannot remain alive for long without food (Hawkes, 1920). Marriner (1926) suggested that the availability of aphids for newly hatched larvae cannot be predicted by the female coccinellid when she lays her eggs.

From these considerations, several authors have reached the conclusion that eating eggs from within their own clutches is beneficial for first instar larvae because it enables them to live longer and so to search more extensively for their aphid prey. This was the conclusion reached by Banks (1956), who found that newly hatched larvae of *Adalia bipunctata* lived, on average, almost twice as long as unfed larvae when they had eaten a single egg of their own species. Larvae fed on two or three eggs lived longer still and succeeded in reaching the second instar within the same amount of time as larvae fed on an excess of aphids.

Dimetry (1974) found that *A. bipunctata* larvae could complete their development to adulthood having consumed an average of 170 eggs in that time. Although cannibals provided with 40 eggs per day had an increased life span, the females laid fewer eggs than those fed with a surplus of aphids. Dimetry considered that larvae were unlikely to depend entirely on eggs to complete their development in a natural situation, so the reduction in fecundity resulting from such a diet would not be important. In agreement with Banks (1956), she considered that egg eating was beneficial in allowing the larvae to develop in the absence of aphids. This was also the conclusion reached by Kaddou (1960) for the case of *Hippodamia quinquesignata* (Kirby), by Kehat (1968) for *Pharoscymnus numidicus*, a coccinellid predator of the date palm scale, *Parlatoria blanchardi* and by Basedow (1982) for *Coccinella septempunctata.*

Brown (1972) reached slightly different conclusions for the coccinellid species *Lioadalia flavomaculata* and *Cheilomenes lunata.* While egg cannibalism did increase the survival of first instar larvae, they became less active after eating eggs. He concluded that although egg eating is unlikely to increase the larvae's ability to search for aphids, it may reduce the number of aphids that they would need to find in order to reach the second instar. Larvae of *C. lunata* were able to reach the second instar after feeding on only two eggs. Pienkowski (1965) also found that larvae of *Coleomegilla maculata lengi* which had eaten at least three eggs before dispersal from their clutches were able to reach the second instar, but were less active and dispersed later than unfed larvae. Pienkowski concluded that although in the temporary absence of food, cannibalism would increase larval survival, it would also tend to reduce their efficiency as predators if aphids or other food was available. This would then diminish or negate any apparent advantages of limited egg cannibalism.

The survival of aphidophagous chrysopid larvae has also been found to increase with egg cannibalism when no other food is available. Elbadry and Fleschner (1965) found that larvae of *Chrysoperla carnea* could complete their development when fed only on eggs of their own species. Bar and Gerling (1985) also reported that *C. carnea* could complete their development when fed only on conspecific eggs. However, 200 eggs or more were needed for a larva to reach pupation and the authors regarded egg cannibalism in this species as a mechanism for enabling survival until normal prey is found, rather than as providing food for complete development. The larvae of *Chrysopa perla*, when fed only on an excess of conspecific eggs, are able to reach the second instar in roughly the same time as larvae fed on an abundance of aphids (Canard, 1970).

Gastrophysa viridula larvae are surrounded by their future food source, the dock leaf, from the moment they hatch out. The argument that egg cannibalism may allow first instar larvae to survive longer and perhaps to search more extensively for their usual food items when these are not immediately available cannot therefore be applied to this species. However, the possibility remains that cannibalistic larvae may have a higher rate of survival, an increased rate of development or increased fecundity when compared to non-cannibals if eating conspecific eggs provides a nutritionally superior food source than dock leaves.

The effects of egg cannibalism on fecundity, developmental rates and survival have been studied quite extensively in flour beetle populations. Stanley, in 1938, reported that egg eating increased the rate of oviposition in *Tribolium confusum.* Later, Rich (1956) and Sonleitner (1961) tried to confirm the connection between cannibalism and reproductive rates in *T. confusum* and *T. castaneum* respectively. They were unable to confirm this relationship, but they did find that females were more voracious egg eaters than males which was considered to be consistent with the idea that egg eating may provide nutrients necessary for egg maturation. Later still, Ho and Dawson (1966) reported that *T. castaneum* individuals that had fed on eggs at the rate of 0.67 eggs per day throughout their larval development laid about 10% more eggs per day as adults than beetles that had not fed on eggs. There were only two replicates per treatment, however, so the results would probably best be regarded as suggestive rather than conclusive.

Mertz and Robertson (1970) found that daily handling of *T. castaneum* larvae resulted in slower development than was found for larvae reared without handling. This effect was most pronounced at high densities and handling also resulted in increased mortality at the highest density. However, they found that feeding handled larvae on conspecific eggs almost completely compensated for the effects of handling and density. Egg eating therefore had a beneficial effect on both developmental rate and survival in this species.

In the chrysomelid beetle, *Labidomera clivicollis*, Eickwort (1973) reported that larvae feeding on conspecific eggs developed more quickly than non-cannibalistic larvae, especially during the early first instar. She speculated that the probability of survival is therefore likely to be greater in the cannibalistic larvae than in the non-cannibals.

There is therefore quite a lot of existing evidence to suggest that cannibalism and egg cannibalism in particular can be beneficial in terms of various components of an individual's fitness. Experiments were designed to determine if egg-eating has similarly beneficial effects in *Gastrophysa viridula.*

METHODS

Survival of larvae fed only on conspecific eggs

An initial experiment was undertaken to find out if *Gastrophysa viridula* larvae are able to complete their development when provided only with eggs of their own species as food. Three clutches of eggs laid by three females from the "Field" laboratory stock were isolated in small, 5-cm. diameter petri dishes lined with moistened filter paper and a piece of dock leaf. On the day before expected hatching, 8 eggs were selected at random from each of the three clutches and placed individually in small petri dishes lined with moistened filter paper. When a larva hatched, the time of hatching was recorded and 6 eggs laid that day were placed in a circle around the larva, taking care not to disturb it. Thereafter, the number of eggs eaten was counted daily under a binocular microscope to the nearest quarter of an egg and the old eggs replaced with newly laid ones. The larvae were at first checked at four-hourly intervals for moulting, but after 4 days when it seemed unlikely that any of the larvae would reach the second instar, the larvae were only checked once daily. The life span, to the nearest day, of each larva was recorded.

Survival, developmental rates and attained adult weights of larvae fed on dock leaves only or dock leaves plus conspecific eggs

A second experiment was carried out to investigate the effects of eating eggs on the developmental rates and survival of larvae. Three clutches of eggs from different "Field" stock beetles were again isolated in small petri dishes. One day before hatching, 22 eggs were taken at random from each clutch. The length and greatest width of each egg was measured under a binocular microscope using a micrometer eye-piece. The eggs were then stored individually on pieces of dock leaf in small petri dishes lined with equal volumes of moistened sterilized sand. Each egg was weighed on a Cahn automatic electrobalance on the morning of the day of hatching. A fine paint brush was used to manipulate the eggs to avoid damaging them. The eggs were then returned to the petri dishes and assigned randomly to one of three treatment groups. This ensured that each group contained eggs of different sizes from each of the three clutches. The time of hatching was recorded to the nearest hour. The three treatment groups were as follows :-

Group A. 22 larvae hatched out in this group. They received only dock leaf as food. The dock leaves were obtained from plants grown under identical conditions in a greenhouse and only the youngest, fully-unfurled leaf was taken from each plant. Circles of dock leaf were cut using cork borers and pastry-cutters. The larvae hatched out on 1 cm. diameter circles of leaf. The leaves were changed on the fourth day after hatching for 2 cm. diameter circles, then again on the seventh day for 2.5 cm. diameter circles and finally on the ninth and twelfth days for 5 cm. diameter circles. This represented an excess of dock leaf for each developing larva, whilst at the same time minimizing any disturbance of the larvae. The filter paper was kept very moist at all times to prevent the leaves from wilting.

Group B. 21 larvae hatched out in this group. They received dock leaf as described for group A and in addition they were given eggs throughout their first instar. Twelve eggs, less than half a day old, were placed around each newly hatched larva in two concentric rings, taking care not to touch the larva. Thereafter, the number of eggs eaten were counted each day under a binocular microscope to the nearest quarter of an egg and the old eggs were replaced daily with 20 eggs laid on the day of change-over. No eggs were given after the larva had reached its second instar.

Group C. 21 larvae hatched out in this group. They were given dock leaf as described for group A and eggs during their first instar as described for group B. Eggs were also given throughout their second and third instars in this group. These eggs were given at the times when the pieces of leaf were changed, placing the new eggs on the new piece of leaf before making the change-over. Forty eggs were given on the fourth day after hatching, 50 on the seventh day and 100 on the ninth and twelfth days after hatching. All eggs had been laid on the same day as the change-over was made. The number of eggs eaten by the larvae was counted daily until the seventh day. Thereafter it was decided only to count the eggs eaten at the time when the old eggs were to be replaced by the new ones in order to minimize disturbance.

The time taken for each larva to reach the second and third instars was checked for at two-hourly intervals. The time taken to reach the pupal stage and adult eclosion was checked for at twelve-hourly intervals. The number of individuals surviving to each of these stages was obtained from these records and the newly emerged adults were weighed on the Cahn electrobalance. One-way analysis of variance, chi-square analyses and Fisher's exact tests were used to analyze the results for any differences between the three groups in developmental rates, survival or adult weight.

Longevity, lifetime egg production and female size

Ideally, I would have liked to check the lifetime egg production of the surviving females from each group when mated. However, I had already gathered data on lifetime egg production in relation to female size while doing an experiment on sperm competition. I anticipated that if any differences in adult weight were found between the three different groups, the data from the sperm competition experiment could be used to judge if this would also imply differences in fecundity of the females that had been reared on the three different diets.

Virgin females were mated with either two normal males (NN), one normal and then one irradiated male (NR), one irradiated male then a normal male (RN) or two irradiated males (RR) (See Chapter 3 for details). Any females that did not mate with a second male could not be included in the sperm competition analysis, but were here included with the NN group of females if the only mating was with a normal male and with the RR group if the mating was with an irradiated male. The females were kept in 9 cm. diameter petri dishes lined with moistened filter paper and supplied daily with fresh dock leaf. The filter paper in each dish was changed at least every five days. The females began to lay eggs between 4 and 7 days before mating took place. The number of clutches laid by each female was checked twice daily until her death and the number of eggs in each clutch was counted. The egg-laying period and the longevity of each female was recorded. After they had died each female's hind tibia was measured under a binocular microscope using a micrometer eye-piece. Results were obtained from 42 females. All manipulations were carried out in a laminar flow cabinet. Analysis of variance and covariance was used to analyze the results.

Pattern of egg numbers laid in successive clutches

Whilst counting the number of eggs and clutches laid by each female, quite a distinctive pattern began to emerge in the numbers of eggs that had been laid in successive clutches. There appeared to be quite a clear pattern of laying alternately higher and lower numbers of eggs in successive clutches. This pattern was usually maintained throughout the major part of the females' egg-laying periods, but became less clear towards the end of their lives when the number of eggs laid per clutch appeared to be generally declining.

One-sample runs tests were used to test for non-randomness in the number of eggs laid in successive clutches by individual females - firstly including all clutches laid over their lifetimes and secondly including only the first 75% of clutches laid in their lifetimes. Each female's mean clutch size was used to divide the data on her clutch sizes into two categories. All values below the mean formed one category and all values equal to or greater than the mean formed the other category. Regression analysis was used to examine the relationship between clutch size and clutch order both for individual females and for the pooled results of all the females - again firstly including all clutches and secondly including only the first 75%.

RESULTS

Survival of larvae fed only on conspecific eggs

None of the larvae that had been given only eggs as food managed to reach the second instar. The mean life span of these larvae was 6.5 ± 0.29 (n = 24). The mean total number of eggs eaten by each larva was 3.8 ± 0.54.

Survival, developmental rates and attained adult weights of larvae fed on dock leaves only or dock leaves and conspecific eggs

One-way analysis of variance confirmed that the eggs from which larvae subsequently hatched did not vary significantly between the three treatment groups for the following measures :- egg length (F=1.659, p=0.199), egg width

(F=0.363, p=0.697) and egg weight (F=0.141, p=0.869), d.f.=2,61 in each case.

Chi-square tests and Fisher's exact tests (where expected values were less than 5) were carried out to establish whether or not survival to the second and third larval instars, pupation and adult eclosion was independent of the group treatment. The results are presented in Table 5.1. The survival of larvae to the second and third instars was not significantly affected by group treatment, but survival to the pupal stage and adult eclosion was. Further chi-square tests established that survival of larvae to these later stages in group B was significantly different from the survival of larvae in groups A and C. The survival of larvae in group A was not significantly different from the survival of larvae in group C. The survival in group B was at least twice as high to these later stages than the survival of larvae in groups A or C.

The results of one-way analyses of variance to test the effects of treatment group on the time taken to reach the second and third instars, the pupal stage, adult eclosion, the duration of the pupal stage and the weights of adults on eclosion are presented in Table 5.2. The mean and standard error for these measures in each group are also presented. The only significant effect of treatment group on any of these measurements was on the time taken to reach the third instar. Duncan's multiple range test established that the time taken by group B larvae to reach the third instar was significantly different at

TABLE 5.1. THE EFFECTS OF TREATMENT GROUP ON THE SURVIVAL OF LARVAE

Treatment group A = dock leaf only
B = dock leaf plus conspecific eggs during first instar
C = dock leaf plus conspecific eggs during all three instars

a) SURVIVAL OF LARVAE TO SECOND INSTAR

i)

Treatment group	Dead	Alive	Fisher's exact test.
A	4	18	$p = 0.302$
B	4	17	

ii)

Treatment group	Dead	Alive	Fisher's exact test.
A	4	18	$p = 0.159$
C	1	20	

iii)

Treatment group	Dead	Alive	Fisher's exact test.
B	4	18	$p = 0.148$
C	1	20	

b) SURVIVAL OF LARVAE TO THIRD INSTAR

Treatment group	Dead	Alive	Chi-square test.
A	11	11	$X^2 = 1.25$
B	7	14	$0.50 < p < 0.75$
C	9	12	

c) SURVIVAL OF LARVAE TO PUPATION

Treatment group	Dead	Alive	Chi-square test
A	17	5	$X^2 = 8.46$
B	9	12	$0.01 < p < 0.025$
C	17	4	

2x2 contingency table for treatment groups A,B. $X^2 = 5.33$, $0.01 < p < 0.025$.
2x2 contingency table for treatment groups A,C. $X^2 = 0.09$, $0.75 < p < 0.90$.
2x2 contingency table for treatment groups B,C. $X^2 = 6.43$, $0.01 < p < 0.025$.

d) SURVIVL OF LARVAE TO ADULT ECLOSION

Treatment group	Dead	Alive	Chi-square test
A	17	5	$X^2 = 10.12$
B	9	12	$0.005 < p < 0.01$
C	18	3	

2x2 contingency table for treatment groups A,B. $X^2 = 5.33$, $0.01 < p < 0.025$.
2x2 contingency table for treatment groups A,C. $X^2 = 0.51$, $0.25 < p < 0.50$.
2x2 contingency table for treatment groups B,C. $X^2 = 8.4$ $0.001 < p < 0.005$.

TABLE 5.2. ONE-WAY ANALYSES OF VARIANCE TO TEST THE EFFECTS OF TREATMENT GROUP ON DEVELOPMENTAL RATES AND ADULT WEIGHT

Treatment group A = dock leaf only
B = dock leaf plus conspecific eggs during first instar
C = dock leaf plus conspecific eggs during all instars

a) TIME (IN HOURS) TO REACH THE SECOND INSTAR

	d.f.	Mean Squares	F	p	Group	n	Mean	S.E.
Between groups	2	10.14	0.47	0.63	A	18	86.1	0.71
Within groups	52	21.59			B	17	84.6	1.12
Total	54				C	20	85.4	1.28

b) TIME (IN HOURS) TO REACH THE THIRD INSTAR

	d.f.	Mean Squares	F	p	Group	n	Mean	S.E.
Between groups	2	1439.18	3.29	0.05	A	11	183.3	8.42
Within groups	34	436.96			B	14	164.1	1.98
Total	36				C	12	181.3	6.93

c) TIME (IN DAYS) TO REACH PUPATION

	d.f.	Mean Squares	F	p	Group	n	Mean	S.E.
Between groups	2	13.55	2.16	0.15	A	5	17.5	1.41
Within groups	18	6.24			B	12	15.5	1.92
Total	20				C	4	18.0	1.62

d) TIME (IN DAYS) TO REACH ADULT ECLOSION

	d.f.	Mean Squares	F	p	Group	n	Mean	S.E.
Between groups	2	9.79	1.88	0.18	A	5	23.2	1.50
Within groups	17	5.20			B	12	20.9	0.55
Total	19				C	3	22.0	0.76

e) DURATION (IN DAYS) OF PUPAL STAGE

	d.f.	Mean Squares	F	p	Group	n	Mean	S.E.
Between groups	2	0.42	1.72	0.21	A	5	5.9	0.24
Within groups	17	0.24			B	12	5.4	0.14
Total	19				C	3	5.5	0.29

f) ADULT WEIGHT (mg.)

	d.f.	Mean Squares	F	p	Group	n	Mean	S.E.
Between groups	2	1.53	0.76	0.48	A	5	9.91	0.75
Within groups	17	2.01			B	12	10.66	0.39
Total	19				C	3	9.79	0.73

the 5% significance level from the time taken by larvae in the other two groups. No other significant differences were found using this procedure. The time taken by group B larvae was shorter than the time taken by larvae in groups A and C.

Although no other significant differences were found, the results of the other one-way analyses of variance all show a similar trend. The mean time taken to reach the second instar, pupal stage and adult eclosion was always shortest for larvae in group B. The mean duration of the pupal stage was shortest for group B larvae and the mean adult weight at eclosion was greatest in group B larvae.

Longevity, lifetime egg production and female size

Analysis of variance and covariance was carried out on the data gathered from females during the sperm competition experiment, taking the following variables as factors:- total number of eggs produced in lifetime, number of clutches produced in lifetime, mean clutch size laid over lifetime, longevity, duration of laying period, mean number of eggs laid per day throughout lifetime (total number of eggs divided by longevity), mean number of eggs laid per day throughout egg-laying period (total number of eggs divided by egg-laying period). The main effect examined was that of group- NN, NR, RN or RR. None of the measures of fecundity or longevity varied significantly according to the type of males the females had mated with. The effect of female size as measured by the hind tibia length was examined as a covariate in the analyses. Once again, the measures of fecundity and longevity did not vary significantly according to female size. These results are presented in Table 5.3. The females laid a mean of 34.4 ± 2.2 clutches in their lifetime. The mean total number of eggs laid was 1383.5 ± 88.5. They lived on average 55.2 ± 3.0 days, although their mean egg-laying period was considerably shorter - 39.6 ± 2.8 days. These figures represent the mean ± S.E. for the 42 females.

Pattern of egg numbers laid in successive clutches

The relationship between clutch size and increasing clutch order showed a negative trend for most of the 42 females when all the clutches were entered into the regression analyses. Eleven of the females showed significantly negative relationships of clutch size with increasing clutch order at the 5% significance level. Eight of the females showed a positive trend in this relationship, but the relationship was not significant in any of these cases. When the results from all the females were pooled together, the regression analysis showed that there was an overall negative relationship between clutch size and clutch order that was significant ($F=66.07$, $p<0.0001$, r.sq.=4.38%, d.f.=1,1442. - see Table 5.4.a).

TABLE 5.3. ANALYSES OF VARIANCE AND COVARIANCE TO TEST THE EFFECTS OF FEMALE SIZE AND THE TYPES OF MALES MATED WITH ON MEASURES OF FECUNDITY AND LONGEVITY

Hind tibia length was taken as a measure of female size. " Group" refers to the type of males mated with - NN, NR, RN or RR - (see text).

a) TOTAL NUMBER OF EGGS PRODUCED IN LIFETIME

	d.f.	Mean Squares	F	p
Covariate				
Female size	1	254338.88	0.76	0.39
Main effect				
Group	3	290710.61	0.87	0.47
Explained	4	281617.68	0.84	0.51
Residual	37	334362.70		
Total	41	329216.84		

b) NUMBER OF CLUTCHES PRODUCED IN LIFETIME

	d.f.	Mean Squares	F	p
Covariate				
Female size	1	97.89	0.51	0.48
Main effect				
Group	3	302.98	1.57	0.21
Explained	4	251.71	1.31	0.29
Residual	37	192.52		
Total	41	198.29		

c) MEAN CLUTCH SIZE OVER LIFETIME

	d.f.	Mean Squares	F	p
Covariate				
Female size	1	7.66	0.38	0.54
Main effect				
Group	3	32.01	1.57	0.21
Explained	4	25.92	1.27	0.30
Residual	37	20.45		
Total	41	20.99		

TABLE 5.3. (continued)

d) LONGEVITY

	d.f.	Mean Squares	F	p
Covariate				
Female size	1	487.47	1.26	0.27
Main effect				
Group	3	228.08	0.59	0.63
Explained	4	292.93	0.76	0.56
Residual	37	386.54		
Total	41	377.41		

e) PERIOD OF LAYING

	d.f.	Mean Squares	F	p
Covariate				
Female size	1	754.25	2.41	0.13
Main effect				
Group	3	418.37	1.34	0.28
Explained	4	502.34	1.60	0.19
Residual	37	313.48		
Total	41	331.91		

f) MEAN NUMBER OF EGGS LAID PER DAY OF LIFE

	d.f.	Mean Squares	F	p
Covariate				
Female size	1	57.86	0.88	0.35
Main effect				
Group	3	22.14	0.34	0.80
Explained	4	31.07	0.47	0.76
Residual	37	65.77		
Total	41	62.39		

g) MEAN NUMBER OF EGGS LAID PER DAY OF EGG LAYING PERIOD

	d.f.	Mean Squares	F	p
Covariate				
Female size	1	185.01	1.81	0.19
Main effect				
Group	3	29.63	0.29	0.83
Explained	4	68.47	0.67	0.62
Residual	37	102.36		
Total	41	99.06		

TABLE 5.4. **REGRESSION ANALYSES TO EXAMINE THE RELATIONSHIP BETWEEN CLUTCH SIZE AND CLUTCH ORDER**

Pooled results from 42 females.

a) THE REGRESSION INCLUDES ALL CLUTCHES OF THE 42 FEMALES

The regression equation is Clutch size = 42.37 - 0.103 clutch order.

Analysis of Variance

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	2891.01	66.07	<0.0001	4.38%
Error	1442	43.76			
Total	1443				

b) THE REGRESSION INCLUDES THE FIRST 75% OF CLUTCHES FROM EACH OF THE 42 FEMALES

The regression equation is Clutch size = 41.12 - 0.001 clutch order which is not significant

Analysis of Variance

Source	d.f.	Mean Squares	F	p	r.sq.
Regression	1	0.23	0.007	0.934	0.001%
Error	1087	32.99			
Total	1088				

c) THE REGRESSION INCLUDES THE LAST 25% OF CLUTCHES FROM EACH OF THE 42 FEMALES

The regression equation is Clutch size = 40.91 - 0.612 clutch order.

Analysis of Variance

Source	d.f.	Mean Squares	F	P	r.sq.
regression	1	1622.17	22.30	<0.0001	5.83%
Error	360	72.75			
Total	361				

TABLE 5.4. (continued)

d) STEPWISE MULTIPLE REGRESSION USING THE LAST 25% OF CLUTCHES FROM EACH FEMALE

Size was taken as the dependent variable; clutch order and the number of clutches constituting 25% of each female's clutches were taken as independent variables.

The regression equation is Clutch size = -0.56 clutch order - 0.06 no.of clutches +41.26. (F=10.17, p=0.0001, r.sq.=5.39%, d.f.=2,357).

Variable	b	S.E. b	t	p
No. of clutches	-0.06	0.16	-0.37	0.712
Clutch order	-0.56	0.15	-3.78	0.0002
(Constant)	41.26	1.45	28.55	<0.0001

When the regression analyses were repeated, using only the first 75% of clutches laid by each female, the majority of females again showed a negative trend in the relationship between clutch size and clutch order but only two of these were significant at the 5% significance level. Seventeen females showed positive trends in this relationship, but none of these were significant. When the results from all the females were pooled together, regression analysis showed that there was no overall significant relationship between clutch size and clutch order over the first 75% of the females' clutches (F=0.01, p=0.934, r.sq.=0.001%, d.f.=1,1089 - see Table 5.4.b)

When regression analyses were performed using the last 25% of clutches of each female, it was found that 30 of the females showed negative trends in the relationship between clutch size and clutch order and 5 of these were significant at the 5% significance level. Nine of the females showed positive trends in this relationship, but none of these were significant at the 5% significance level. Three of the females produced too few clutches for the last 25% to be analyzed by regression analysis. When the results from all the females were pooled together, regression analysis showed that there was a negative relationship between clutch size and clutch order over the females' last 25% of clutches that was significant (F=22.30, p<0.0001, r.sq.= 5.83%, d.f.=1,360 see Table 5.4.c).

To check that this overall negative relationship was not simply due to females that laid more clutches having smaller clutch sizes, a stepwise multiple regression was carried out, taking clutch size as the dependent variable and clutch order and the number of clutches constituting 25% of each female's clutches as independent variables. The regressin equation is Clutch size = -0.56 clutch order -0.06 number of clutches + 41.26. (F=10.17, p=0.0001, r.sq.=5.39%, d.f.=2,357). The relationship between clutch size and the number of clutches was not significant (b=-0.06, t=-0.37, p=0.712). The relationship between clutch size and clutch order remained significantly negative (b=-0.56, t=-3.78, p=0.0002. see Table 5.4.d).

The results of the one-sample runs tests on the females' successive clutch sizes are presented in Table 5.5. When all of the clutches were included in the analyses, 23 of the 42 females showed significantly non-random patterns of the numbers of eggs they laid in successive clutches caused by higher numbers of runs than would be expected if the sequential distribution of clutch sizes was random. Three of the females produced too few clutches to be able to determine the significance of a one-sample runs test. The results from only one of the females produced as many runs as there were clutches laid.

However, when only the first 75% of each female's clutches were considered, 7 females showed perfect alternation of clutch size from above or equal to their mean clutch size to less than the mean clutch size throughout

TABLE 5.5. ONE-WAY RUNS TESTS TO TEST FOR NON-RANDOMNESS IN THE CLUTCH SIZE OF SUCCESSIVE CLUTCHES OF INDIVIDUAL FEMALES

a) All clutches included					b) First 75% of clutches included				
No. of clutches	Mean clutch size	Runs	z	p	No. of clutches	Mean clutch size	Runs	z	p
50	38.4	47	6.02	<0.0001	38	39.3	37	5.43	<0.0001
53	41.1	45	5.07	<0.0001	40	41.9	37	4.99	<0.0001
41	41.2	37	4.81	<0.0001	31	41.3	29	4.40	<0.0001
37	41.7	33	4.53	<0.0001	28	41.8	25	3.85	<0.0001
37	37.1	32	4.19	<0.0001	28	37.9	28	4.82	<0.0001
36	37.1	32	4.26	<0.0001	27	38.4	27	4.72	<0.0001
31	41.2	28	4.11	<0.0001	23	41.3	21	3.43	0.0006
27	43.8	26	4.33	<0.0001	20	44.6	20	3.91	<0.0001
42	41.5	35	4.00	0.0001	32	41.9	27	3.56	0.0004
21	38.7	21	4.05	0.0001	16	39.2	16	3.36	0.0008
66	39.5	48	3.65	0.0003	50	40.8	41	4.36	<0.0001
24	40.8	22	3.55	0.0004	18	40.8	16	2.67	0.008
37	42.7	30	3.34	0.0008	28	44.0	28	4.82	<0.0001
34	37.3	27	3.25	0.001	26	38.6	24	3.86	0.0001
23	39.0	20	3.13	0.002	17	39.4	16	3.03	0.002
31	37.4	25	3.00	0.003	23	37.4	20	3.13	0.002
13	40.7	13	2.94	0.003	10	39.7	10	2.35	0.019
44	42.2	32	2.91	0.004	33	42.4	27	3.26	0.008
57	37.8	40	2.84	0.005	43	38.6	33	3.22	0.001
30	39.9	23	2.45	0.014	23	40.2	22	3.86	0.0001
25	43.6	20	2.46	0.014	19	43.7	14	1.43	0.152
28	41.7	21	2.28	0.023	21	41.7	18	2.70	0.007
33	41.3	24	2.13	0.033	25	42.6	21	2.87	0.004
47	49.8	30	1.80	0.073	35	49.8	23	1.90	0.058
20	44.1	14	1.73	0.084	15	44.3	14	2.71	0.007
39	41.1	25	1.55	0.120	29	41.4	16	0.34	0.734
21	42.1	12	1.07	0.284	16	41.9	6	0.00	1.000
49	49.9	27	0.85	0.397	37	50.2	21	0.46	0.649
37	38.7	12	0.28	0.779	28	39.4	11	0.87	0.386
31	38.2	16	0.00	1.000	23	38.4	12	0.00	1.000
35	42.1	4	-3.98	0.0001*	26	44.4	26	4.60	<0.0001
44	41.2	8	-3.49	0.0005*	33	44.2	22	2.00	0.045
42	35.2	11	-3.25	0.001*	32	36.8	21	1.52	0.129
29	43.9	4	-2.82	0.005*	22	45.1	16	2.05	0.042
32	39.8	7	-1.96	0.050*	25	40.0	5	-2.29	0.022*
66	35.0	27	-1.52	0.129	50	38.0	21	-0.78	0.436
45	37.8	16	-1.10	0.270	34	40.4	22	1.32	0.187
41	33.3	16	-1.04	0.300	31	35.2	12	-0.86	0.390
29	40.8	12	-0.04	0.967	22	41.0	6	-1.42	0.157
8	40.4	6	~~~~	~~~~~	6	41.2	5	~~~~	~~~~~
5	19.4	4	~~~~	~~~~~	4	19.8	3	~~~~	~~~~~
3	38.3	2	~~~~	~~~~~	2	38.5	2	~~~~	~~~~~

* indicates a significantly non-random result because of too few runs.

~~~ indicates that the significance could not be calculated.
~~~

their lives. Of the results from the 39 females to which the one-sample runs test could be applied, 26 females (66.7%) showed non-random sequential clutch sizes resulting from a higher than expected number of runs.

Clutch size in most females therefore seems to alternate about a constant value throughout the greater part of their egg-laying lives, declining only towards the end of this period.

DISCUSSION

Survival of larvae fed only on conspecific eggs

The fact that larvae were not able to reach the second instar when given only conspecific eggs as food suggests that the nutritional content of these eggs is not sufficient, on its own, for larval development. However, the larvae in this experiment were reared in isolation. Long (1953, 1955), working with the butterfly, *Pieris brassicae*, found that larvae reared in groups of 60 spent 25% more time feeding and developed faster than larvae reared singly.

As discussed in the introduction to this chapter, the eggs of several predacious coccinellids and lacewings have been shown to provide sufficient nutrition to allow larval cannibals to develop at least to the second instar. Apart from Pienkowski's (1965) observations on *Coleomegilla maculata lengi*, the larvae were in all cases reared singly. But although the eggs of the coccinellid species discussed are laid in clutches, the larvae disperse after hatching in search of their prey and so feeding singly is probably not a problem for these beetles. Similarly, the lacewings lay their eggs singly and the larvae search on their own for their prey, so being reared singly in experiments is unlikely to cause problems in these species either.

However, the larvae of *Gastrophysa viridula* remain in groups while feeding on the dock leaves until their third instar. It would therefore be necessary to repeat the feeding experiment, raising the larvae in groups rather than singly. In this way it might be possible to determine whether their inability to reach the second instar was due to their eating too few eggs because of lack of stimulation when feeding alone - or if the eggs themselves provide insufficient nutrition for their development or the larvae are reluctant to eat sufficient quantities of eggs, even when feeding in groups.

Survival, developmental rates and attained adult weights of larvae fed on dock leaves only or dock leaves and conspecific eggs

The results of the second experiment suggest that there are benefits to be gained from being cannibalistic in terms of developmental rate and probability of survival, at least for cannibals eating eggs during their first instar. The results from group C larvae, however, at first appear to be contradictory to this idea. These larvae fed on eggs not only during their first instar, but also throughout their second and third instars. Yet they showed poorer survival rates than the B group larvae fed on eggs only during their first instar and they did not survive any better than larvae fed only on dock leaves in the A group. The B group larvae consistently did better than the C group larvae in all the other measurements taken where benefits of egg-eating might have been expected to show up, even though all but one of these effects were non-significant.

I was therefore prompted to try to find an explanation for these apparently strange results. A two-sample t-test was carried out to check if the larvae in groups B and C ate significantly different numbers of eggs during their first instar. This was not the case. Group B larvae ate, on average, 1.6 ± 0.3 eggs ($n = 21$) during their first instar. Group C larvae ate, on average, 1.9 ± 0.2 eggs ($n = 21$) during this same period - ($t=0.8$, $p=0.43$, $d.f.=39$). The difference in survival rates was therefore not due to group C larvae eating fewer eggs during their first instar than group B larvae.

After carrying out an experiment that showed that when larvae are presented with eggs of different ages, the quantity of eggs eaten is negatively related to the age of the eggs (see Chapter 4), I began to suspect that the larvae in group C had inadvertently been provided with eggs that, rather than having beneficial effects on their development and prospects of survival, may actually have been harmful. Since Pasteels *et al.* (1986) found that the eggs of *Gastrophysa viridula* contain an isoxazolinone glucoside compound that is toxic to ants, the results of the egg-age experiment suggest that the toxicity of the eggs may increase with age and that the eggs may be toxic not only to ants but also to *G. viridula* larvae themselves.

In the light of these results, it seemed worthwhile checking to see if any eggs more than two days old had been eaten by group C larvae. This was not possible during the first instar because throughout this period the eggs were changed every day for ones that had been laid on the same day as the change-over. But to avoid causing extra disturbance to the C group larvae during their second and third instars, the eggs were only changed at the same time as the leaves were being changed in all the groups. This meant that eggs were left for periods of two or three days before changing them.

Unfortunately, again to minimize disturbance, the number of eggs eaten were only counted daily during the first period of the second instar when the eggs were left for three days before changing them. But even from these incomplete records, it is evident that some older eggs had been eaten by the larvae. Of the three group C larvae that survived to adult eclosion, two had not eaten eggs more than two days old during the second instar when the eggs eaten had been counted daily. These larvae had eaten a total of 8.5 and 8.75 eggs each during their second and third instars and a total of 10.5 and 9.25 eggs respectively throughout their entire lives. The third larva that survived to adult eclosion had eaten one egg more than two days old during the daily counting period and had eaten a total of 17.5 eggs throughout its second and third instars and a total of 20.5 eggs throughout its whole life. However, of the twelve larvae that survived to the seventh day after hatching but did not reach the adult stage, all but two had eaten eggs more than two days old during the daily counting period of the second instar, eating on average 1.0 ± 0.1 (n = 10) of these older eggs during that time.

The results from this experiment are therefore consistent with the view that eating older eggs may have detrimental effects on the survival and developmental rates of *Gastrophysa viridula* larvae. However, it must be stressed that direct evidence of this possibility has not yet been obtained. Instead of drawing any firm conclusions from the results of the C group larvae, I would rather view them as having led to the formulation of a hypothesis that it will be important to test in the future.

One further possibility that should be considered in connection with the disparity of the results between group B and group C larvae is that larvae in group C may have been subjected to more disturbance than group B larvae. Mertz and Robertson (1970) found that daily handling of *T. castaneum* larvae reduced their developmental rates and increased mortality at high densities in comparison with unhandled larvae. However, precautions were taken to try to avoid extra disturbance of group C larvae in comparison with the other two groups. The group C larvae were possibly disturbed more than group B larvae when the eggs eaten during the second instar were at first counted daily. But this only involved placing each petri dish under a binocular microscope without actually touching the larvae, so it would seem unlikely that such a small amount of disturbance could have caused such a strong difference in survival rates between group B and group C larvae.

The benefits gained by individuals through cannibalism have important consequences for the evolution of this behaviour. Hamilton (1964a) stated the conditions that are expected to be fulfilled if "altruistic" or "selfish" behaviour are to be selected for. Cannibalism is a very clear example of selfish behaviour in which one animal reduces the fitness of another, to the extreme extent of taking its life, in order to gain in terms of its own individual fitness. But, according to Hamilton (1964a), this behaviour is only likely to be selected for if the gain in fitness to the cannibal is sufficient to outweigh the loss of any copies of the causative gene or genes that may have been shared by both the cannibal and its victim.

Eickwort (1973) presented a modified version of Hamilton's model to analyze the evolution of cannibalism through kin selection. Hamilton (1964a) had stated that the inclusive fitness of an individual will be increased, and the selfish behaviour selected for if $-k < 1/r$. For the purpose of analyzing cannibalistic behaviour in particular, Eickwort (1973) took k to represent the change in fitness of the victim divided by the gain in fitness of the cannibal, and *r* is the coefficient of relatedness between the two individuals. She expressed *k* as $-p / p'$, where *p* is the probability of survival to adulthood and *p'* is the increase in the cannibal's probability of survival.

The benefits in terms of survival gained by group B cannibals were certainly great enough under experimental conditions to fulfill Eickwort's criterion, even for the case of larvae eating full-siblings ($0.227/0.344 < 2$). However, larvae don't normally live in such artificially controlled conditions and without field data on survivorship of cannibals and non-cannibals it is not possible to say with any certainty that the benefits of cannibalism of young eggs, at least, will always outweigh the costs of eating the victim. But the fact that advantages of cannibalism can be detected in fairly favourable laboratory conditions suggests that it is quite likely that it will pay larvae at the very least to eat unrelated eggs under natural conditions.

If it does pay larvae to eat conspecific eggs, it would not be surprising to find that females have evolved means of avoiding having their eggs eaten by unrelated cannibals. The position in which females lay their eggs in relation to previously laid clutches may be important in this respect (see Chapter 6). As already mentioned, the evidence presented in Chapter 4 suggests that older eggs are in some way protected against cannibalism and since eggs have been shown to be toxic against potential predators such as ants, there is good reason to suspect that the protective mechanism against cannibalism may also be one of chemical defence. If this is the case, females would gain extra protection for their eggs against inter-clutch cannibalism, but the situation of intra-clutch cannibalism would have to be looked at in a different light.

Larvae do eat viable eggs from within their own clutches (see Chapter 2) and these eggs are either full-siblings or half-siblings (see Chapter 3). If the benefits from cannibalism are great enough to outweigh the loss of such close relatives, then this result would not be surprising. But even if it pays larvae to eat closely related young eggs, if older eggs are toxic it surely would not pay larvae to eat those that are close to hatching within their own clutches and females would be expected to have been selected to minimize the risks of this happening.

If eating older eggs is in fact detrimental to the fitness of larvae, the question of why any of these eggs should be eaten at all still remains. One possibility is that the larvae may be unable to distinguish between old and young eggs. Although larvae have been shown to eat greater quantities of younger than older eggs, this distinction may be based on the levels of defence chemicals inside the eggs (see Chapter 4 for discussion). If this is the case, no distinction can be possible until at least one egg has been punctured. This may explain why a very small number of viable eggs are sometimes eaten from within clutches.

No significant differences were found in the adult weights attained by cannibalistic and non-cannibalistic larvae. But since the sample sizes for groups A and C were very small, it would not be reasonable to draw any conclusions from this result.

Longevity, lifetime egg production and female size

No evidence was obtained from these experiments to suggest that cannibalism may be beneficial in terms of adult fecundity or longevity. Adult body size has been found in many insects and other invertebrates to be correlated with both life span and egg production (e.g. Charnov *et al.*, 1981). Contrary to this expectation, however, no evidence was obtained to suggest that longevity or any measures of fecundity were affected by adult size in *Gastrophysa viridula.* Even if cannibalistic larvae were found to result in larger adults than non-cannibals when larger sample sizes were obtained, there is no evidence to suggest that the cannibals would have any fitness advantages over non-cannibals because of their larger size.

Pattern of egg numbers laid in successive clutches

The fact that many females lay a very distinctive sequentially alternating pattern of larger and smaller clutches throughout most of their egg-laying periods was an interesting finding. I am unable to offer any explanations as to how or why this happens. I dissected mature females with the intention of trying to count the number of ovarioles present in the ovaries, but I was unable to see anything except a mass of larger and smaller yellow eggs. No doubt this was partly due to my lack of experience in dissection, but I have since been advised that it would have been better to dissect females before they have actually matured any eggs.

Although only two-thirds of the females showed significantly non-random patterns of sequential clutch sizes when the first 75% of clutches were considered, the one-sample runs test was a fairly stringent one, especially as it took the mean clutch size of those first 75% of clutches of each female as the cut-off point to divide the clutch sizes into two categories. Most of the females did show the distinctive pattern of consecutively larger and smaller clutches during at least part of their egg-laying period, but in some cases this was not of long enough duration to produce a significant result from the one-sample runs test.

That many females do lay in such a distinctive pattern poses some interesting problems for the calculation of "an optimal clutch size" - these females can never lay one absolute optimal clutch size. The difference in consecutive clutch sizes was in some cases as many as ten eggs, but was usually less than that. Nevertheless, a female that lays consecutively 45, 35, 45 .... eggs does not fit with any present predictions of optimal clutch sizes.

It seems possible that females may not be able to control their clutch sizes quite so finely as theoretical models are able to predict they should! It may not then be necessary to seek selectively adaptive explanations for why some viable eggs are eaten from within clutches. Perhaps the best that females can do, given that they may in some way be constrained to lay different numbers of eggs in alternate clutches, is to reach some compromise between laying clutch sizes that minimize the risk of viable eggs being eaten and maximizing the number of eggs per clutch to minimize search costs.

The finding that the clutch size laid by females does not decline, except towards the end of their egg-laying lives, fits with the predictions of Parker and Courtney (1984). They predicted that where females gather resources for egg maturation during the adult stage and the maximum number of clutches that can be laid is independent of clutch size, the clutch size laid by females will not be affected by a fixed mortality risk between laying of clutches that is independent of age. On the other hand, they predicted that in species where resources for egg production are gathered during the larval stages thereby setting a limit to the maximum number of eggs that could be produced in a lifetime, the clutch size should decline throughout the females' lives when there is a fixed risk of death between clutches. *Gatrophysa viridula* females gather food resources for egg maturation throughout their egg-laying lives. They therefore fit Parker and Courtney's predictions for clutch size in insects showing that particular feature of their life history.

CHAPTER 6

INTER - CLUTCH CANNIBALISM IN *G. VIRIDULA*

Outline of Chapter 6

In this chapter some aspects of inter-clutch cannibalism are examined. In the first section an experiment is described which was designed to test the effect of distance between a group of newly hatched larvae and a clutch of unhatched eggs on the extent of inter-clutch cannibalism. The period for which larvae of *Gastrophysa viridula* remain potentially cannibalistic is the subject of the second section and finally, in the third section, the possibility that larvae may differentiate between kin and non-kin eggs as subjects for cannibalism is explored.

6.1. THE EFFECT OF DISTANCE BETWEEN CANNIBALISTIC LARVAE AND EGG VICTIMS ON THE EXTENT OF INTER-CLUTCH CANNIBALISM

INTRODUCTION

Gastrophysa viridula larvae are cannibalistic on eggs from other clutches as well as eating eggs from within their own clutches. This was confirmed in the laboratory. Newly laid eggs were placed close to clusters of hatching larvae on dock leaves and the larvae were seen to attack the eggs. First instar larvae were also found eating eggs from clutches that had been placed close to them on dock leaves during field experiments.

If inter-clutch cannibalism was potentially a cause of high egg mortality, females would be expected to protect their eggs not only against within-clutch cannibalism, but also against cannibalism by larvae from other clutches. It seemed that one possible way for females to avoid this type of predation might be for them to place their eggs in a position where the risks of being encountered by cannibalistic larvae would be minimized. My first thoughts were that females might benefit by choosing to lay their eggs on leaves that did not already have eggs on them. Field observations had shown that larvae do not usually move off the leaf on which they hatch until at least the beginning of the second instar. Since I wasn't at that time sure for how long larvae remain cannibalistic and I had only ever seen first instar larvae cannibalizing eggs, I thought that by laying eggs on egg-free leaves, females would ensure at least some degree of protection against inter-clutch cannibalism for their eggs.

A preliminary experiment did not bear this idea out. Potted dock plants, from which all but two leaves had been removed, were enclosed in net bags. One female beetle was placed on each plant and when she had laid a clutch of eggs, she was replaced by a second female. The leaf on which each female had laid her clutch of eggs was noted. In five cases out of ten, the second female laid her clutch on the same leaf as the clutch of the first beetle; in the other five cases, she laid her eggs on the other leaf. Since they showed no preference for which leaf to lay their eggs on, it did not seem worthwhile pursuing that idea any further.

However, I decided to look more closely at what happens when eggs are placed on a leaf where a clutch of eggs has already been laid. In particular, I was interested to see if the risks of cannibalism diminish with distance from the earlier laid clutch. This was investigated by placing groups of eggs at set distances from clutches of eggs already laid on dock leaves.

METHODS

This experiment was carried out in a temperature-controlled greenhouse (22 °± 2°C.) and since it took place in April and May, natural day-length lighting conditions were used. Dock plants were reared in six inch plant pots in the greenhouse. They were sown at the same time from the seeds of a single plant and were therefore as similar to each other as could be controlled for.

A female beetle of the "Field" laboratory stock was enclosed in a net bag on a leaf of each of forty plants. The leaf chosen in each case was the second youngest. The youngest leaf is a curled leaf in the centre of the plant. As the leaves grow in a rosette formation (see Cavers & Harper, 1958), it is quite easy to tell the age order of the leaves.

Once a female had laid a clutch of eggs, both she and the net bag were removed from the plant. The eggs were allowed to develop on the leaf and were then watched closely on the day of expected hatching. As soon as the eggs showed signs of darkening, a group of 15 eggs was placed on the leaf, close to the original clutch, using a moistened paintbrush. These introduced eggs had been laid on the previous day by beetles from the "Roadside" stock. The eggs are covered in a tacky substance and so can be fixed quite easily to the leaf's surface. Each group of 15 eggs was assigned randomly to a position in which the edge of the group was either 0.5 cm. or 1 cm. away from the edge of the nearly-hatching clutch of eggs, and either directly above or below the clutch (parallel to the leaf's axis) or to the right or to the left of it.

The time of the start of hatching was noted for each clutch and the number of hatched larvae was counted. At four-hourly intervals after the eventual start of hatching, the leaf was checked for signs of the larvae having come in contact with the eggs. The movements of the larvae are such that a four-hourly interval is usually sufficient to ensure that all contacts would be seen. However, a double-check was possible in that, as first instar larvae move across the leaf, they are at the same time feeding on it and so they leave a trail of where they have been. Larvae at this stage remain tightly aggregated in groups and it is very rare for any of them to wander away from the body of the group. The trail they leave is therefore a reliable indication of their previous whereabouts. A note was made of the time at which first contact between the larvae and the eggs was observed. The number of eggs in the introduced group that had been attacked was checked with the aid of a magnifying glass at the end of each 24-hour period after the start of hatching. In cases where slugs or spiders interfered with the eggs or larvae, the observations on that plant were discontinued and the results were not included in the analysis of the experiment. All observations were ended four days after hatching. By this stage, the larvae had mostly entered the second instar and some were beginning to move off the leaf on which they had hatched.

In all, 14 sets of observations were obtained for the 0.5 cm. group; 3 in which the introduced eggs were above the hatching larvae, 3 below, 5 to the right and 3 to the left. Seventeen sets of results were obtained from the 1 cm. group; 4 above, 3 below, 5 to the right and 5 to the left.

A two-sample t-test was used to compare the mean times taken by the larvae in the 1 cm. group and the 0.5 cm. group to contact the introduced eggs. Analysis of variance and covariance was used to examine the effects of the various factors measured during the experiment on the number of the introduced eggs eaten.

RESULTS

The results of the two-sample t-test showed that the time taken to first come into contact with the introduced eggs was significantly shorter for the 0.5 cm. group larvae than for the 1 cm. group larvae. For the analysis, the time of first contact was taken to be the mid-point between the observation time at which this was first noted and the previous observation time. The time taken by the 0.5 cm. group larvae to make contact with the eggs was 2.3 ± 0.3 hours ($n = 14$ groups) and the time taken by the 1 cm. group larvae was 29.7 ± 4.9 hours ($n = 13$ groups) - ($t = -5.62$, $p < 0.0001$, d.f. = 12.1). The 0.5 cm. group larvae had made contact with the eggs within 4 hours after hatching in all cases except one. In this case the larvae had come into contact with the eggs within 8 hours after hatching. In the 1 cm. group the time to first contact ranged from within 12 hours after hatching to within 68 hours after hatching. It is worth noting, therefore, that by the end of the first day after hatching, in all the 0.5 cm. group tests the larvae had come into contact with the introduced eggs, but this was true in only eight of the seventeen 1 cm. group tests. Even after four days, there were still 4 cases among the 1 cm. tests where the larvae had not come into contact with the eggs at all. These cases could not, of course, be included in the two-sample t-test but they reinforce the conclusion that distance between larvae and eggs has an important effect on the time taken by the larvae to reach the eggs.

Analysis of variance and covariance was used to examine the effects of the following variables on the number of introduced eggs attacked:- distance between the introduced eggs and the hatching larvae; the position of the eggs relative to the larvae, whether above, below, to the right or to the left; the number of larvae that hatched in each test and the time taken to first come in contact with the eggs.

As the data for the number of eggs eaten one day after hatching were not normally distributed because of the high number of zero values in the 1 cm. group, these results could not be analyzed by analysis of variance and covariance. It can only be noted that in all 14 cases in the 0.5 cm. group, the introduced eggs had been attacked within 24 hours of the larvae hatching. In the 1 cm. group the eggs had been attacked in only 2 cases within this same time period.

The most important results to look at are those for the total number of eggs that had been attacked by the end of four days after hatching. Except for the four cases in which the introduced eggs were not encountered at all, the larvae in all the 1 cm. group tests had come in contact with the eggs within three days after their hatching. The analysis of variance and covariance of these results is presented in Table 6.1.1.b. The data for the number of eggs eaten, the number of larvae hatching and the time taken to encounter the eggs had first of all been tested for normal distribution using the Kolmogorov-

TABLE 6.1.1. ANALYSIS OF VARIANCE AND COVARIANCE TO EXAMINE THE EFFECTS OF DISTANCE BETWEEN THE INTRODUCED EGGS AND THE OLDER CLUTCH OF EGGS, POSITION OF THE INTRODUCED EGGS, THE NUMBER OF LARVAE HATCHED FROM THE OLDER EGGS AND TIME TAKEN FOR LARVAE TO CONTACT THE INTRODUCED EGGS ON THE NUMBER OF INTRODUCED EGGS EATEN

The distance was either 0.5cm. or 1 cm.
The position was either above, below, to the right or to the left of the older clutch.

a) KOLMOGOROV-SMIRNOV GOODNESS OF FIT TEST FOR NORMALITY

	K-S z	p
Number of eggs eaten	0.883	0.417
Number of larvae	0.540	0.933
Time	1.299	0.068

b) ANALYSIS OF VARIANCE AND COVARIANCE

	d.f.	Mean Squares	F	p
Covariates				
Number of larvae	1	51.18	2.78	0.114
Time	1	185.70	10.09	0.006
Main effects				
Distance	1	122.35	6.65	0.020
Position	3	6.45	0.35	0.789
Interaction				
Distance x Position	3	0.52	0.03	0.993
Explained	9	39.31		
Residual	17	18.41		
Total	26	25.64		

Covariate	Raw regression coefficient
Number of larvae	0.150
Time	-0.152

Smirnov goodness of fit test. The results of this test are shown in Table 6.1.1.a.

The position of the eggs and the number of eggs that hatched out in each test had no significant effect on the numbers of eggs attacked. However, both the effects of distance and the time taken to first encounter the eggs were highly significant. Of these two effects, the time taken to first encounter the eggs was the stronger. The regression coefficient for the effect of time is negative, indicating that the greater the time taken to encounter the eggs, the fewer the number of eggs that were attacked.

The mean number of eggs attacked in the 0.5 cm. group tests was 8.9 ± 1.3 (n = 14). This was higher than in the 1 cm. group tests (3.4 ± 1.1; mean ± S.E. for 13 tests). This analysis excluded the cases in which the larvae did not encounter the eggs at all. The mean number of eggs attacked in the 1 cm. group tests was therefore even lower when these four cases were taken into account (2.6 ± 0.88; n = 17 tests).

Even though the results for the cases in which the larvae did not come across the introduced eggs could not be included in the analysis of variance and covariance, their occurence does strengthen the conclusion that the effects of distance and time taken to encounter the eggs was important in determining the number of eggs eaten.

DISCUSSION

The results indicate that eggs laid only one centimetre away from a previously laid clutch of eggs will be afforded quite a considerable degree of protection against cannibalism from the larvae hatching out from the older clutch. This is at least true for eggs that are laid on the day before the older eggs are due to hatch.

Factors affecting the number of eggs eaten in the two groups

The number of eggs cannibalized was affected both by the distance between the introduced eggs and the hatching larvae, and by the time taken for the larvae to encounter the eggs. The fact that eggs that were placed half a centimetre away from the previously laid clutch were in all cases encountered within eight hours of the larvae hatching is not surprising in view of the particular pattern of movements taken by larvae in the first few hours after hatching. When larvae first hatch, they are very tightly packed, covering roughly the same area of leaf as did the clutch of eggs from which they hatched. They then tend to move in such a way as to be less tightly aggregated and to cover a larger area of leaf. But the area of leaf they cover is still roughly circular and the centre of the circle remains in roughly the same position. Any eggs that have been laid within this area of expansion, irrespective of their position relative to the central point of this area, are therefore bound to be encountered and vulnerable to attack. This was probably the case for eggs laid half a centimetre away from the hatching larvae.

The mean time taken for larvae to reach eggs in the 1 cm. group was more than twelve times as great as the mean time taken by the 0.5 cm. group, even though the larvae were only at two times the distance from the eggs as was the case in the 0.5 cm. group. The greater time taken and the greater variability in the time can again probably be accounted for by the pattern of movements taken by larvae after they have expanded to occupy a larger circular area. After this expansion stage, the larvae move, as a group, away from the position of the original clutch of eggs. If any unhatched eggs are outwith the limits of the expansion area of the larvae, the time taken for them to be encountered will depend on the direction initially taken by the larvae and the position of the eggs with respect to the larvae. If the eggs lie in the same direction as taken by the larvae, they will be encountered much more quickly than if they lie in some other direction. Larvae do not continue indefinitely in the direction in which they initially set off on, and so eggs lying in other directions may eventually be encountered. However, as seen from the results of this experiment, there is a chance that some of these eggs will not be encountered at all.

It is important to note, though, that the difference in the number of eggs cannibalized in the two groups was not simply a reflection of the difference in the probability of the larvae encountering the eggs. The analysis of variance and covariance only included cases in which the larvae did come into contact with the eggs. Nevertheless, the effects of distance between larvae and eggs and of time taken to encounter the eggs were still highly significant.

Possible reasons why eggs laid at a greater distance than others from a previously laid clutch suffer less cannibalism

There are two possible reasons why the eggs in the 1 cm. group tests suffered less cannibalism than the eggs in the 0.5 cm. group tests, even when encountered. Larvae reaching eggs in the 1 cm. group tests were older than those encountering eggs in the 0.5 cm. group. If larvae remain cannibalistic for only a limited period after hatching, the probability of the eggs being attacked would depend on the age of the larvae, and in the experiment this was roughly the same as the recorded time of first contact with the eggs. The period during which larvae remain cannibalistic after hatching is examined in the next section. It was found that larvae, given the opportunity, will eat conspecific eggs during all three instars. The age of the larvae would not, therefore, seem to be the factor accounting for the difference in the extent of cannibalism in the two groups.

A second possibility is that the risk of being attacked may depend on the age of the eggs. The 1 cm. group eggs would nearly always have been older than the 0.5 cm. group eggs at the time when they were first encountered by the larvae. Unfortunately, the age of the eggs cannot be more precisely specified. When the experiment was planned, it was not anticipated that the age of the eggs might be a very important factor which should be taken into account. The introduced eggs had always been laid on the day before the older clutch of eggs hatched out. This seemed a sufficient measure to take to ensure that the introduced eggs were all of a similar age. However, when it became clear from the results that the age of the eggs may affect the extent of the inter-clutch cannibalism, I realized that it would have been a good idea to have controlled more finely for the age of the eggs. Nevertheless, the results did suggest the possibility that the eggs may have some intrinsic form of protection against cannibalism that is dependent on the age of the eggs. This idea was explored in Chapter 4. It was found that the quantity of eggs eaten by first instar larvae, when the eggs were placed immediately around the hatching larvae, was negatively related to the age of the eggs.

It does not seem surprising, then, that female beetles showed no preference for laying their eggs either on a leaf that already had a clutch of eggs on it or on one that did not. It seems quite likely that a female could gain a high degree of protection for her eggs against cannibalism from larvae hatching from a clutch of eggs already on a leaf by laying her eggs outwith a certain range surrounding the older clutch. This range is likely to be greater than 1 cm., but could depend on the difference in the ages of the two clutches of eggs. To have the same level of protection, eggs could be laid closer to relatively young eggs than to relatively old eggs. The time taken to encounter the unhatched eggs would be greater for larvae hatching from the relatively old eggs than for larvae hatching from the relatively young eggs. But the eggs laid next to the young eggs would have had more time to develop their intrinsic protective mechanism before the young eggs hatched. If females are unable to tell the age of eggs already present on a leaf, they would be expected to lay their own eggs at a distance from them that would minimize the risks of cannibalism, making the assumption that the previously laid eggs are on the point of hatching out.

Experimental observations on the positions in which females lay their eggs

Along with the experiment described in the introduction to this section, several others were carried out to see if females preferred to lay eggs on leaves without eggs rather than on leaves with eggs already present. In one of these experiments, single females were enclosed by net bags on potted dock plants that had all but two leaves removed. The females were allowed to lay several successive clutches. The positions and time of laying of these clutches were recorded twice daily. Again, no evidence was found that females show any preference for laying their eggs either on leaves where they had previously laid eggs or on egg-free leaves. But, in the cases where more than one clutch of eggs had been laid on a leaf, the distances between each clutch and all the others on the leaf could later be calculated.

The position of each clutch had been recorded as a pair of co-ordinates. The axis of the leaf was used as the Y-axis, and an imaginary line at right angles to the Y-axis, passing through the centre of each clutch was used as the X-axis. Five leaves were obtained with more than one clutch of eggs. Two of the leaves had 2 clutches, two had 3 clutches and one had 4 clutches. The lengths of the leaves ranged from 12.9 cm. to 14.5 cm. and their maximum widths ranged from 6.1 cm. to 8.9 cm. The mean distance between a clutch and any other clutch on the same leaf was 4.4 ± 0.4 cm (n = 14). The distances ranged from 1.8 cm. to 7.4 cm., but it is interesting to note that the two clutches separated by only 1.8 cm. had been laid on consecutive days. The larvae hatching from the older clutch would have had a maximum of 24 hours in which to reach the unhatched eggs of the younger clutch. The chances of this happening have been shown to be less than 50% when the distance between clutches is 1 cm. It is likely that the chances of this happening would be even lower when there is a gap of 1.8 cm. between the clutches. In addition, the eggs of the younger clutch would already have developed quite a high degree of intrinsic protection by the time the eggs of the older clutch hatched.

The available evidence suggests that females may be able to minimize the risks of their eggs being cannibalized by larvae hatching from previously laid clutches on the same leaf. They could achieve this by laying their eggs outwith the range in which there is a likelihood of their being encountered by the earlier hatching larvae. Even if there remained a small risk of the eggs being encountered, the time taken for the larvae to reach the eggs would ensure that the eggs could have developed a high degree of intrinsic protection in that time. The evidence suggests that individual females, at least, may lay their eggs at "safe" distances from their previously laid clutches when they are confined to laying them on only two leaves. Females are not so rigidly confined in natural situations. They will, however, come across leaves with clutches of eggs laid by other females. They would then have a choice of laying their eggs at a "safe" distance from the previously laid eggs or moving to a new leaf. If the chances of finding a leaf with no eggs on it was small, searching for such a leaf would entail considerable time costs. The female's best strategy might then be to lay her eggs at an appropriate "safe" distance from the previously laid eggs, even if there was a very small risk of a very small proportion of her eggs being cannibalized. These are possibilities that seem worthwhile investigating more fully in the future.

6.2. THE PERIOD DURING WHICH LARVAE REMAIN CANNIBALISTIC

INTRODUCTION

As discussed in the previous section, the time for which larvae remain cannibalistic after hatching might have been important in determining the extent to which inter-clutch cannibalism occurs. If larvae are cannibalistic for only a short, limited period after hatching, this could have explained why eggs contacted after this time were not attacked. A simple experiment was designed to test for cannibalistic tendencies in larvae of different ages from the day of hatching through to the third instar.

METHODS

Clutches of eggs laid by females from the "Field" laboratory stock were stored individually in small, 5 cm. diameter petri dishes lined with moistened filter paper and a piece of fresh dock leaf. On the days of expected hatching, the petri dishes were checked hourly and the time of hatching of each clutch was noted. The larvae were then maintained in 9 cm. diameter petri dishes for periods ranging from 0 - 192 hours from the time when hatching was first noticed. During this time they were kept well supplied with an excess of fresh dock leaves.

During the test period, eggs were collected daily from "Roadside" laboratory stock female beetles kept individually in petri dishes. Each morning, the petri dishes were cleared of all previously laid eggs, and only eggs laid after this time were used in the experimental tests. The eggs used were therefore unrelated to the larvae and were always less than eight hours old.

Ten replicate tests were carried out for each group of larvae 0, 24, 48, 72, 96, 120, 144, 168 and 192 hours after hatching. For the 0-hour group, sixteen larvae were transferred to a 5 cm. diameter petri dish lined with moistened filter paper and containing a 2.5 cm. diameter circle of fresh dock leaf, cut with a cork borer. Sixteen eggs that had been laid that day were placed in the centre of the piece of dock leaf and the larvae were arranged around the eggs.

Twenty-four hours after the introduction of the larvae, the eggs were examined under a binocular microscope and scored in the manner described on the following page :-

Proportion of each egg eaten (x)	Score
Not eaten	0
$0 < x \leq 0.25$	0.125
$0.25 < x \leq 0.5$	0.375
$0.5 < x \leq 0.75$	0.625
$0.75 < x \leq 1$	0.875

A total score was obtained for each group of sixteen eggs.

Larvae 24 and 48 hours after hatching were tested in the same way. Larvae 72, 96 and 120 hours after hatching were also tested in a similar way, except that the petri dish contained a larger piece of dock leaf, cut to the size of the dish with a pastry-cutter. Larvae 144, 168, and 192 hours post-hatching were tested in 9 cm. diameter petri dishes with the piece of dock leaf being 8 cm. in diameter. The size of the dock leaf was chosen so that the eggs at the centre would always be encountered by the test larvae whilst ensuring that the leaf was never completely eaten by the end of the test period. At the end of each test, larvae were either just older than 1, 2, 3, 4, 5, 6, 7, 8 or 9 days old. By the end of their ninth day, larvae had entered a pre-pupation stage and had become inactive and stopped eating. The experiment was ended at this point.

All manipulations of eggs and larvae were carried out in the sterile conditions of a laminar flow cabinet. Regression analysis was used to test for any increase or decrease in the score of eggs eaten with increasing age of the larvae.

RESULTS

Larvae remained cannibalistic throughout all three instars. No significant increase or decrease in the amount of eggs eaten was found with increasing age of the larvae (Figure 6.2.1). However, the highest number of cases in which the maximum score of 14 was achieved are found in the tests in which the larvae were just over eight days old at the end of the test period. It is possible that if greater numbers of eggs had been used, some difference might have been found in the numbers of eggs eaten by younger and older larvae. But it is certainly safe to conclude that the cannibalistic capacities of larvae did not decline with age. For certain periods during the day preceding moulting between instars, the larvae became inactive and stopped eating. This is not reflected in uniformly low scores for larvae of particular ages. For part of the day around the time of moulting the larvae would have been active and eating and, although the larvae within one set of ten replicates were all of the same age, they did not all reach the second and third instars at the same time.

***FIGURE 6.2.1.* THE RELATIONSHIP BETWEEN THE SCORE OF EGGS EATEN AND THE AGE OF THE LARVAE**

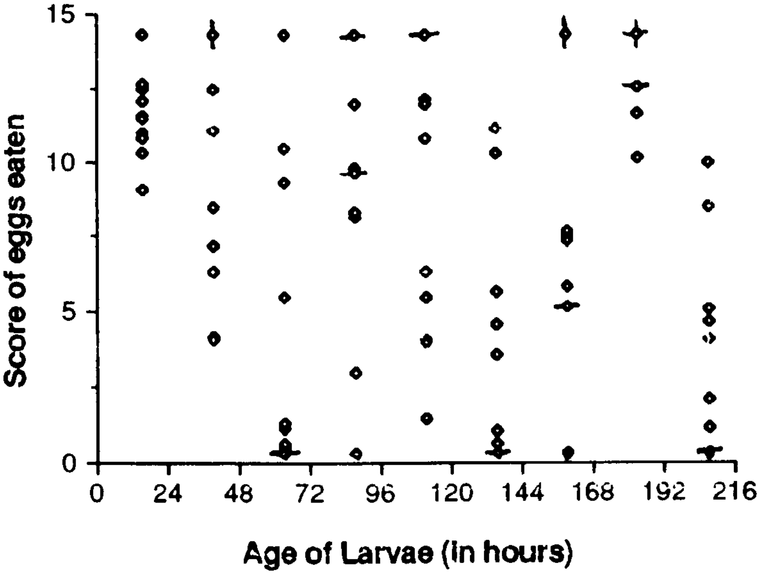
-◇- = 2 data points ◇ = 3 data points ✧ = 6 data points

The regression equation is Score of eggs eaten = 9.02 - 0.01 Age of larvae.

This is not significant

ANALYSIS OF VARIANCE

Source	d.f.	Mean squares	F	p	r.sq.
Regression	1	40.38	1.71	> 0.1	1.9%
Error	88	23.64			
Total	89				


15
10
5
0
Score of eggs eaten
0
24
48
72
96
120
144
168
192
216
Age of Larvae (in hours)

DISCUSSION

These results indicate that it is unlikely that the age of the larvae was the factor that prevented eggs from being attacked, even though they were encountered, in the experiment described in the last section. The fact that green dock leaf beetles remain potentially cannibalistic throughout the three larval instars implies that the risk of inter-clutch egg cannibalism could be very high. During field observations, I noted that by the second and third instars, larvae have usually left the leaf on which they hatched and are moving quite widely between several dock leaves. Any unhatched eggs anywhere on these leaves would potentially be at risk.

But, as shown in the previous section, eggs that are placed only one centimetre away from a previously laid clutch of eggs are afforded a high degree of protection against cannibalism. The most likely explanation for this seems to be that the eggs develop an intrinsic protection mechanism which becomes more effective with age. This finding implies that even though larvae may come across many clutches of eggs during their second and third instars, only clutches containing very young eggs are likely to suffer any great losses through cannibalism.

Females therefore have two potential defences against inter-clutch cannibalism. By laying eggs at an appropriate distance from previously laid clutches, they can probably avoid cannibalism from first instar larvae to a large extent. The position they lay their eggs in is unlikely to provide protection from second and third instar larvae. But since eggs apparently have their own age-dependent protective mechanism against cannibalism, there is probably only a short period during which clutches of eggs are vulnerable to large losses through cannibalism.

The larvae in this experiment had no previous experience of eating eggs before being presented with young eggs during the test period. It would be interesting to find out if larvae that had previously been exposed to older eggs would be deterred from eating other older eggs that they later encountered. It would be especially interesting to find out if exposure to older eggs would deter larvae from eating *any* eggs they later came across, whether they were young or old.

The defensive chemicals that Pasteels *et al.* (1986) found in the eggs of *G. viridula* were only found in the interior of the eggs and not in the gluey substance on their exterior. If these chemicals are involved in the protection of eggs against cannibalism as well as against potential predators such as ants, then at least one egg would have to be punctured for a larva to be exposed to the deterrent properties of the eggs. The effect of sucking the contents of an older, possibly toxic egg, could be sufficient to deter a larva from trying to attack any future eggs. Since larvae would have to puncture an egg before knowing whether it was "nasty" or not, they might avoid attempting to eat any eggs at all.

In Chapter 4 it was seen that even in the case of larvae being presented with 108-hour eggs, more eggs were attacked than could be accounted for by the number of non-viable eggs. This is suggestive that the deterrent properties of the eggs in a clutch is dependent on at least one of them being punctured. It remains to be seen, however, if older eggs are actually toxic to larvae and if the larvae will be deterred from eating any future eggs once they have sucked out some of the contents from one of these older eggs.

6.3. THE EFFECT OF THE RELATIONSHIP BETWEEN CANNIBALS AND THEIR EGG VICTIMS ON THE EXTENT OF INTER - CLUTCH CANNIBALISM

INTRODUCTION

So far, two possible ways of reducing inter-clutch cannibalism have been discussed - the position in which females lay their eggs in relation to previously laid clutches and the eggs' own intrinsic protective mechanism. Another factor that could influence cannibalism of eggs from other clutches would be the possession of a kin recognition system. If larvae were able to distinguish kin from non-kin eggs and refrained from eating any to which they were closely related, inter-clutch cannibalism could again be reduced.

According to the ideas of Hamilton (1963, 1964a, 1964b), animals are expected to behave in such a way as to maximize their inclusive fitness. Since close relatives are more likely to share copies of any rare alleles through common descent than distant relatives, the benefit or detriment of a particular action in terms of inclusive fitness will depend on the relationship of the interacting individuals. This being the case, Hamilton (1964b, 1987) stated that, in certain circumstances, an ability to discriminate between animals of different degrees of relatedness could be beneficial to inclusive fitness and would be expected to evolve. This would be the case, for example, if such discrimination ensured that aid-giving behaviour was directed to close relatives rather than to more distant ones.

In recent years, many examples have been found of animals that are able to distinguish kin from non-kin. In nests of the primitively eusocial bee, *Lasioglossum zephyrum*, the bee guarding the entrance to the nest will allow related nestmates to enter but will not admit unrelated conspecifics (Michener, 1974). Extremely fine levels of discrimination have been found in some animals such as the honey bee, *Apis mellifera*, where different patrilines of half-siblings resulting from multiple inseminations of the queen tend to segregate when a colony swarms (Getz *et al.*, 1982). Discrimination even to the level of individual recognition has been found in invertebrates. Examples include mate recognition as well as parent-offspring recognition in the desert woodlouse, *Hemilepistus reaumuri* (Linsenmair & Linsenmair,1971; Linsenmair, 1972), and discrimination among unfamiliar queens of differing fecundity levels in the fire ant, *Solenopsis invicta* (Fletcher & Blum, 1983).

The list of examples of species in which a kin recognition system has been shown to exist is impressive and growing all the time. Cases have been found where, even though an ability to discriminate between kin and non-kin has been demonstrated, this ability seems to have no apparent effect on inclusive fitness. Tadpoles of the Cascades frog, *Rana cascadae*, can distinguish their full-siblings from both maternal and paternal half-siblings, but any function of this ability is unknown (Blaustein & O'Hara, 1982).

Hamilton (1987) noted that an ability to discriminate between close and distant relatives is likely to be most developed when there is a high cost involved in misplacing acts. The cost in terms of inclusive fitness of eating a full-sibling or half-sibling is potentially very high since this act reduces the relative's reproductive potential to zero.

Following this type of reasoning, several authors (e.g. Blaustein *et al.*, 1987a, 1987b and Wilson,1987) have taken the view that cannibalistic animals will increase their inclusive fitness by feeding preferentially on non-kin. Some examples have been found that substantiate this idea. Wade

(1980) found that avoidance of sibling cannibalism developed in inbred populations of the flour beetle, *Tribolium confusum*, but did not develop in populations with random mating. Individuals in an inbred population will have quite a high proportion of genes in common. It is therefore to be expected that the costs of killing another member of such a population are likely to be correspondingly high. Another example of animals discriminating between kin and non-kin as victims of cannibalism is that of the guppy, *Poecilia reticulata*. Females cannibalize unrelated fry in preference to related fry (Loekle *et al.*, 1982).

However, the development of an ability to discriminate between different levels of kin may depend on whether or not it pays in terms of inclusive fitness to eat closely related individuals. Hamilton's kin selection criterion (1963, 1964a , 1964b) has most often been used to analyze "altruistic" behaviour, but as discussed in previous chapters, it can equally be applied to the problem of selfish behaviour, such as cannibalism. Hamilton stated that the inclusive fitness of an individual will be increased and the selfish trait selected for, if $-k < 1/r$. Eickwort (1973) proposed a modified version of this criterion, specifically to investigate the case of cannibalistic behaviour. The change in fitness of the egg eaten divided by the gain in fitness of the cannibal is represented by k, and r is the coefficient of relatedness.

If this criterion is met for cannibals eating close relatives such as full-siblings as well as unrelated individuals, a kin recognition system might not be beneficial. Discriminating cannibals would miss opportunities to increase their inclusive fitness through eating siblings and could be at a selective disadvantage as compared to non-discriminating cannibals.

On the other hand, situations may exist in which a cannibal could gain in terms of inclusive fitness by eating non-relatives, but the cost of killing a close relative would not be sufficiently compensated for by personal gain - i.e. when the criterion is reversed ($-k > 1/r$). In such cases an ability to distinguish between close and distant or non-relatives could be advantageous and might be expected to evolve.

The results of a laboratory experiment described in Chapter 5 suggested that *G. viridula* larvae may gain benefits in terms of developmental rates and survival, at least when young conspecific eggs are eaten. However, it is not known if such benefits would outweigh the losses incurred through killing a close relative. In section 1 of this chapter it was seen that larvae will eat unrelated eggs from other clutches. They will also eat viable eggs from within their own clutches as seen in Chapter 2. These observations, by themselves, give no indication as to whether cannibalistic larvae make any distinction between kin eggs or non-kin eggs. A more controlled experiment was needed to test this possibility. An experiment was therefore designed to find out if larvae showed any difference in the extent of their cannibalism when presented with either full- and half-sibling eggs or non-related eggs.

METHODS

Clutches of eggs were obtained from laboratory stock beetles originating from two different sources. One stock was obtained from the usual "Field" site at Lancaster University. The other stock originated from the "Roadside" plot, outside Lancaster. *Gastrophysa viridula* is a poor disperser (Zwoelfer, 1973; Potts and Vickerman, 1974). The adults do not fly and although both adults and larvae may move between the plants within a plot, movement over distances greater than forty to fifty metres is unlikely (Whittaker *et al.*, 1979). Since the two sites were five miles apart, individuals from the two stocks were sure to be unrelated.

Female beetles from both stocks were kept individually in 9 cm. diameter petri dishes lined with moistened filter paper and supplied with a piece of dock leaf. On the days when eggs were to be collected, eggs already laid in the petri dishes were removed and the old dock leaf was replaced with a new piece. The petri dishes were then checked every half-hour. As soon as a freshly-laid clutch of eggs was discovered the time was noted and two lots of 16 eggs were taken from it with a moistened paintbrush. Each lot was placed in a 5 cm. diameter petri dish lined with moistened filter paper and dock leaf cut to the size of the container with a pastry-cutter.

One lot was maintained in the laboratory at 20° ± 1°C., with 16h:8h light:dark photoperiod until hatching. The second lot was taken immediately to a cold storage room where the conditions were 4° ± 1°C. and 12h:12h photoperiod. When hatching of the first lot had proceeded to the point where all the larvae had reached the stage of having their head and thorax protruding from the egg shell, the larvae were presented with the 16 eggs of a second lot that had been stored in the cold-room. The eggs were arranged in a circle, immediately around the larvae. The eggs were either from the same clutch as the larvae or from a clutch laid by a beetle from the unrelated clutch. If the two lots were from unrelated clutches, both clutches had been laid within half an hour of each other.

Larvae and eggs from the "Field" stock were labelled "A" and "a" respectively and similarly, larvae and eggs from the "Roadside" site were labelled "B" and "b". Four arrangements of larvae and eggs were obtained in this way. The larvae in Aa and Bb arrangements were either full- or half-siblings of the eggs presented to them (see Chapter 3). The larvae in Ab and Ba arrangements were not related to the eggs given to them. Since 16 A or B larvae were not always obtained in each trial, the number that did hatch out was noted and this was taken into account when analyzing the results.

Twenty-four hours after the eggs had been presented to the larvae, they were examined under a binocular microscope and scored in the same way as described in the methods of section 2 of this chapter. In all, 14 Aa, 19 Bb, 21 Ab and 20 Ba arrangements were tested. Analysis of variance and covariance was used to examine the effects of the number of larvae that hatched and the relationship between eggs and larvae on the score of eggs eaten. Once again, all counting and manipulation of eggs was done under sterile conditions in a laminar-flow cabinet to minimize the risk of fungal infection on the eggs.

A control experiment was designed to test the effect of the conditions in the cold storage room on the development of the eggs. Eggs from the "Field" stock beetles were used. Two lots of 16 eggs from each of seven clutches were treated as described above until hatching of the first lot had proceeded to the stage where the larvae had head and thorax protruding from the egg-shells. The incubation time for each first lot of eggs was noted. At that point the second lot of eggs was returned to the laboratory from the cold-room. The time taken for these eggs to reach the hatching stage was recorded. The means of the incubation times for the first lots of eggs and the means of the times taken for the second lots of eggs to reach hatching after being returned to the laboratory were compared using a two-sample t-test.

The effect of the cold storage room on the viability of the eggs was also checked during the control experiment. The numbers of larvae that hatched from the first and second lots of 16 eggs were noted and the means for the two lots were compared using a two-sample t-test.

RESULTS

The development of the eggs placed under the cold storage room conditions was not completely halted. The incubation time for eggs stored in the laboratory was 120 ± 0.85 hours (n = 7 observations). The time required to reach hatching by eggs stored in the cold room once they had been returned to the laboratory was 115.1 ± 1.0 hours (n = 7 observations) - ($t=3.6$, $p < 0.005$, d.f.=12). The eggs presented to larvae were therefore equivalent to eggs that had been laid a few hours previously. The viability of eggs was not affected by being placed in the cold storage room. The mean number of larvae hatching out from the groups of 16 eggs stored in the laboratory was 15.4 ± 0.3 (n = 7 observations). This was not significantly different from the number of larvae that hatched out from the groups of eggs first stored in the cold room (15.0 ± 0.5, n = 7 observations) - ($t= 0.75$, $p=0.47$, d.f.=10).

Figure 6.3.1 shows the effects of the number of larvae that hatched and the relationship between eggs and larvae on the score of eggs eaten. Analysis of variance and covariance showed that there was a significant relationship between the number of larvae and the score of eggs eaten. The greater the number of larvae, the higher the score of eggs eaten. However the genetic relatedness between eggs and larvae did not affect the score of eggs

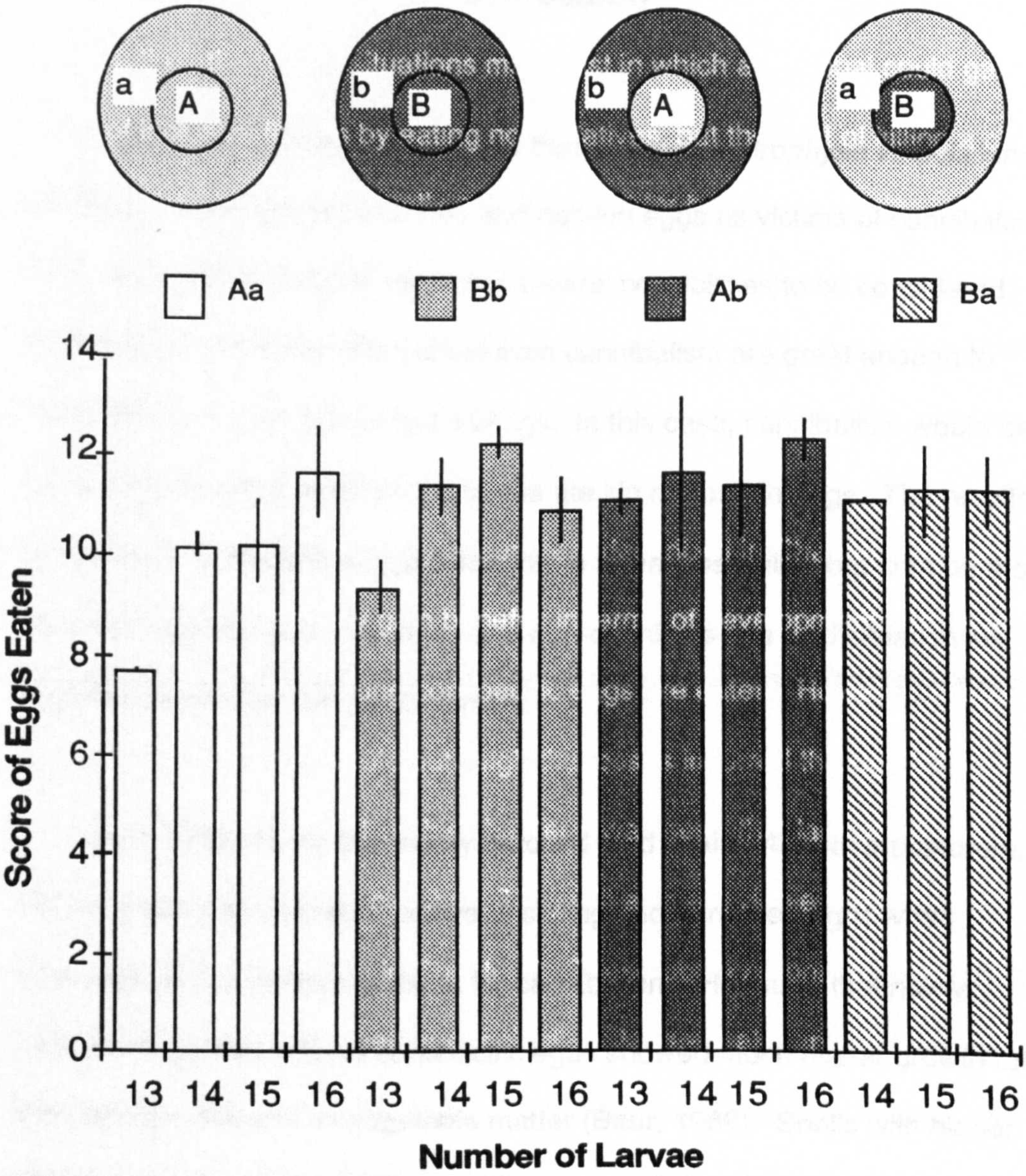
FIGURE 6.3.1. **THE EFFECTS OF THE NUMBER OF LARVAE THAT HATCHED AND RELATEDNESS BETWEEN LARVAE AND EGGS ON THE SCORE OF EGGS EATEN**

The pairs of concentric circles represent the arrangements of larvae (inner circles) and eggs (outer circles). In the Aa and Bb arrangements the larvae and eggs were from the same clutch. In the Ab and Ba arrangements the larvae and eggs were from different clutches and were unrelated. The histogram shows the mean ± S.E. of the score of eggs eaten for each arrangement of eggs and larvae when different numbers of larvae hatched out.

ANALYSIS OF VARIANCE AND COVARIANCE

	d.f.	Mean Squares	F	p
COVARIATE				
No. of larvae	1	13.47	4.16	0.045
MAIN EFFECT				
Kinship	3	4.60	1.42	0.244
Explained	4	6.82	2.11	0.089
Residual	69	3.24		
Total	73	3.43		

COVARIATE	RAW REGRESSION COEFFICIENT
No. of larvae	0.443


a
A
b
B
b
A
a
B
Aa
Bb
Ab
Ba
Score of Eggs Eaten
0
2
4
6
8
10
12
14
13
14
15
16
13
14
15
16
13
14
15
16
14
15
16
Number of Larvae

eaten. These results indicate that first-instar larvae on the first day after hatching do not distinguish between recently laid sibling and unrelated eggs as victims for cannibalism.

DISCUSSION

The results provide no evidence that day-old *Gastrophysa viridula* larvae make any distinction between kin and non-kin eggs as victims of cannibalism. In the light of these results there are several possibilities to be considered. It is possible that the benefits gained from cannibalism are great enough to outweigh the loss of full- or half-siblings. In this case, cannibalism would be selectively favoured whether the larvae ate kin or non-kin eggs. The results presented in Chapter 5 suggest that this is a real possibility, but data on the survival probabilities of cannibals and non-cannibals in a field situation are required before this can be confirmed.

Baur (1987) found that newly hatched land snails, *Arianta arbustorum*, did not make any distinction between sibling and unrelated eggs when presented with a mixture of these for cannibalism. He found that newly hatched snails fed only on conspecific eggs showed much higher growth rates than siblings fed only on vegetable matter (Baur, 1986). Snails with higher growth rates may therefore have a higher probability of survival. In addition, he found that adult size was positively correlated with juvenile growth rate and that fecundity was positively correlated with adult size (Baur, 1984). Using field data on the survival of *A. arbustorum* from egg to maturity and the results of his experiments on the fitness gains of cannnibals over non-cannibals, he concluded that cannibalism may be selectively favoured, even when the victims are full- or half-siblings.

Tepedino and Frohlich (1984) used glass tubes to simulate nests of the alfalfa leafcutting bee, *Megachile rotundata.* Bees were forced to emerge behind either siblings or non-siblings whose development had been delayed by 4 or 17 days. An emerging bee could choose to destroy their nest-mate to get out of the tube or to circumvent the nest-mate or to wait until the nest-mate had emerged before emerging itself. When the development of the nest-mate had been delayed by 4 days, the emerging bees most often waited. When development of nest mates was delayed by 17 days, the emerging bees either circumvented them if possible, or destroyed them. The emerging bees therefore avoided killing their nest-mates unless suicide was the only alternative. But they made no distinction between killing siblings or non-siblings, so kin-recognition was not found in this species either. Using Hamilton's criterion, the authors calculated that in nests of 5 or fewer offspring, destruction of both siblings and non-siblings would be beneficial. There would therefore be no selective advantage for the development of a kin recognition system. However, it is not known how frequently siblings and non-siblings are found in the same nest in nature. If this were an uncommon situation, a kin recognition system would be superfluous anyway.

The frequency with which cannibals are likely to encounter siblings should also be considered for the case of *Gastrophysa viridula*. The only known time when larvae are certain to encounter their siblings or half-siblings is when they have just hatched out. But intra-clutch cannibalism has been shown to be of extremely limited extent. Females appear to lay clutches of such a size that precludes the common occurrence of intra-clutch cannibalism of viable eggs. When it does occur, only a very few viable eggs are eaten (see Chapter 2). Depending on the laying strategy of the females, it is conceivable that first-instar larvae may normally not come across conspecific eggs except in their own clutches. Mothers may lay their eggs "out of reach" of first instar larvae hatching from their previously laid clutches - either by laying them at a great enough distance from their previously laid eggs on the same leaf, or by laying them on a different leaf altogether. These ideas need to be tested in the field, but from the presently available evidence, they seem not unreasonable possibilities. If first instar larvae are unlikely to encounter sibling eggs after leaving their clutch, there would be no need for them to have a kin recognition system.

However, larvae move off the leaf on which they hatched, usually once they have reached the second instar. At that stage, and especially during the third instar, the larvae range quite widely across different leaves of a plant. It would then be quite possible that they could come into contact with sibling eggs. There are then several further possibilities to be considered. One possibility is that older larvae may have powers of discrimination not found in first-instar larvae. This was not tested during this experiment.

A second possibility is that larvae are able to distinguish kin from non-kin in older eggs, but not in one-day old eggs. This might be the case if the larvae used a phenotype matching system as the basis for kin recognition. A phenotype matching system is one in which an animal can learn aspects of its own characteristics or those of close relatives and can then recognize such characteristics in others. Phenotype matching depends on phenotype similarity being well correlated with genotype similarity (Holmes and Sherman, 1982). If *G. viridula* larvae were able to learn cues from the unhatched eggs in their own clutch, they might later be able to use these cues as a template by which to judge whether other eggs they encountered were kin or non-kin. But, if the cues differ in older eggs and younger eggs, larvae having only experience of older eggs in their own clutch would be unable to distinguish kin from non-kin in younger eggs.

But such a system would only have been likely to have evolved if larvae do come across sibling eggs frequently, and if it pays larvae to eat non-related eggs, but not to eat closely related eggs. In this respect, it is important to remember that the eggs of *G. viridula* have been shown to develop an intrinsic protective mechanism against cannibalism. This becomes effective in eggs that are only two days old (see Chapter 4). The protective mechanism has so far only been shown to deter larvae from eating older eggs. But if the mechanism is based on chemical defence, as there are good reasons for suspecting may be the case (see Chapter 4), then eating older eggs may actually be detrimental to the fitness of the cannibalistic larvae.

This idea needs direct verification, but there is some indirect evidence to support it. Larvae that had eaten eggs more than two days old along with younger eggs and dock leaf did less well in terms of developmental rate and survival than larvae fed only on younger eggs and dock leaf. The possibility that the larvae given older eggs were also subject to an increased amount of disturbance that may have had detrimental effects has not been entirely ruled out. However, this possibility seems unlikely to account for such a strong difference in the survival rates (see Chapter 5). If eating older eggs was detrimental to the fitness of larvae, then a kin recognition system would be inappropriate. Rather, a mechanism enabling the recognition and avoidance of all older eggs, no matter whether they were kin or non-kin, might be expected to have evolved.

It would only, then, be advantageous for larvae to eat very young eggs. Females lay, on average, less than one clutch of eggs per day under favourable laboratory conditions (see Chapter 5). The chances of larvae encountering eggs laid by their own mother that are still very young are probably quite low. If that were the case, a kin recognition system would again be superfluous. Field data on the frequencies with which larvae do actually encounter sibling eggs of a young age are required to clarify this point.

CHAPTER 7

GENERAL DISCUSSION

In this chapter a brief report is given of work carried out as part of the research project but not described in detail elsewhere. The main findings of the thesis are brought together and their possible evolutionary significance is discussed. Potentially fruitful areas of future research are suggested.

Summary of work not described in detail elsewhere

As mentioned in the introduction to the thesis, a large-scale field experiment was undertaken during the first summer season of the project. The aim of the experiment was to investigate the relationship between the number of eggs laid per unit area of dock leaf and the subsequent development and survival of the resulting larvae. *Rumex obtusifolius* plants were grown from seed in a greenhouse and planted out in an area of grassland at Ness Gardens, Cheshire, in the spring of 1985. They were planted in 14 groups of four, with each plant positioned at the corner of a notional square of side length 1.5m. A cage was formed around each plant by hammering four wooden stakes into the ground surrounding the plant and covering this frame with fine mesh netting. Each cage was tied at the top to allow access to the plant and anchored at the bottom by placing the netting in a trench around the plant and replacing the sods of earth on top.

Female *Gastrophysa viridula* were allowed to lay eggs on plants, each one enclosed on a leaf by a small net bag. Each plant within a group of four was randomly assigned to receive either 50, 100, 200 or 400 eggs. Accordingly, females were left on the plants until eggs had been laid in excess of the numbers required. The females and the excess eggs were then removed. The number of leaves on each plant and an estimate of their area were obtained at the beginning of the experiment and were monitored regularly throughout the experiment.

However, having carefully controlled the numbers of eggs per plant, the activity of slugs then modified these numbers. Slugs travelling across leaves removed eggs in their mucous slime trail. On 30 plants, all eggs had been removed before they had a chance to hatch. Half of the remaining plants had between 1 and 19 eggs and only five had egg numbers greater than 50. The highest number of eggs on a plant was 89.

The original aim of the experiment could not therefore be carried out. However, it was decided to make use of the experimental set-up to study the movements of the larvae on the plants. Each plant with larvae on it was visited once or twice daily and records were made of the numbers and positions of larvae. Each leaf on a plant was numbered according to its age and the positions of first and second instar larvae were recorded as pairs of coordinates on particular leaves. The axis of the leaf was used as the Y-axis, and an imaginary line at right angles to the Y-axis, passing through the centre of each clutch was used as the X-axis.

The positions of third instar larvae were recorded only as the particular leaf on which they were situated. Third instar larvae moved extensively and singly, rather than in groups and too much disturbance could make them drop from the leaf onto the ground. First and second instar larvae were found to move on average approximately 16mm. per day, in groups. They stayed on the leaf on which they had hatched for periods of between three and seven days. The reason for leaving the leaf was not depletion of the leaf area since generally only a small percentage of the leaf was eaten. Larvae had a tendency to move onto younger leaves after they had moved off their original leaf.

It was during this field experiment that it was discovered that cannibalism is a feature of the life history of *G. viridula*. Intra-clutch cannibalism was suspected from the counts made of numbers of eggs before hatching and the corresponding numbers of larvae found after hatching. Since the activities of slugs could conceivably have produced the same result, verification of intra-clutch cannibalism was obtained by observing the hatching of clutches of eggs under a binocular microscope. Microscope observations also enabled the developmental stages of the eggs to be identified and subsequent observations indicated the possible involvement of fully developed, viable eggs in cannibalism.

Counts of overall cannibalism rates were carried out both on clutches laid on living plants and on clutches laid on cut leaves in petri dishes. An initial experiment was then set up to try to estimate the percentages of viable and non-viable eggs eaten from within clutches. *Rumex obtusifolius* plants were grown from seed in pots. A female beetle was enclosed in a net bag around each of 60 leaves and was allowed to lay a single clutch of eggs before being removed. The number of eggs in each clutch was counted. Half of the clutches were allowed to proceed normally through hatching. The numbers of resulting larvae were counted. In the remaining clutches, each newly-hatched larva was carefully removed with a fine paintbrush before being able to cannibalize any eggs. The numbers of resulting larvae were again counted along with the numbers of non-viable eggs remaining.

Unfortunately, the hatching of clutches in this experiment was abnormal. In all clutches there was a very high rate of non-hatching, with more than 50% of eggs failing to hatch in many cases. The cause of such hatching abnormalities was not known, but as a consequence the results from this experiment were of no use in estimating the percentages of viable and non-viable eggs cannibalized in normal clutches. Even if the clutches had had normal hatching, it was found that there were problems associated with this method such as accidentally knocking eggs off the leaf when removing newly-hatched larvae. A more satisfactory method was developed and this is described in Chapter 2.

Although the field experiment had brought to light the existence of intra-clutch cannibalism, experiments were undertaken to determine if inter-clutch cannibalism was also a possibility. First of all, an experiment was carried out in which 30 transparent sandwich boxes were lined with moist blotting paper and supplied with a cut dock leaf. A female was placed in each box and allowed to lay a clutch of eggs. In half the boxes the original female was allowed to lay a second clutch of eggs and in the remaining boxes a different female laid a second clutch of eggs.

The number of eggs in each clutch was counted. The position and the time of laying and hatching of each clutch were also recorded. Any signs of attack on the second clutch of eggs by larvae from the first clutch were looked for. Very few of the second clutches were attacked. However, this experiment served to indicate factors which might be important in determining the existence or extent of inter-clutch cannibalism.

Such factors were thought to include the distance between the two clutches, the time interval between the laying of the two clutches and the direction of the second clutch with respect to the first. A second experiment was therefore carried out, controlling for the time between the laying of the two clutches and the relative direction of the second clutch with respect to the first, but varying the distance between the two clutches. This experiment was carried out in petri dishes with newly-hatched larvae placed at varying distances on a piece of cut leaf from newly-laid eggs. The experiment indicated that the distance between the two clutches was important in determining the extent of inter-clutch cannibalism. However, the set-up in petri dishes was very artificial and it was decided to pursue these studies, but using plants grown in the field for the experimental set-up.

First of all, an attempt was made to control the position of the two clutches laid on a leaf of a plant. A female was enclosed in a specially constructed very small cage which could be clipped onto the leaf at the required position. Although this resulted in fairly accurate positioning of clutches, it also resulted in areas of leaf being eaten immediately around the clutches. This would have hindered the movements of larvae when they hatched. It was therefore decided to allow a female to lay a clutch of eggs freely on a leaf. Her feeding was then generally not close to where she had laid her eggs. The second clutch of eggs was then placed by hand in the required position as described in the first section of Chapter 6.

Since it was found that distance was an important factor in determining the extent of inter-clutch cannibalism, it was decided to look again at the movements of larvae on dock plants. Although larval movements had been observed during the field experiment, most of the clutches had been severely reduced in numbers so that only a few larvae hatched and moved together on the leaves. From the point of view of inter-clutch cannibalism it was desired to know the speed with which larvae from complete clutches move, and if they show any directional preferences in their movements after hatching.

For this purpose , females were allowed to lay clutches of eggs on the leaves of potted dock plants in a green-house, one clutch per leaf. The movements of larvae were plotted every six hours after hatching. However, the mechanism which normally should have functioned to maintain the green-house at a fairly constant temperature apparently was not working properly at this time. During the day, temperatures rose dramatically and resulted in wilting of the dock leaves, even when they were watered frequently. The movements of the larvae on these leaves could not then be considered as "normal".

A series of experiments was carried out to investigate the oviposition decisions of females. An experiment was designed to investigate whether or not females will alter the number of eggs they lay in each of their clutches or the total number of clutches that they lay when presented with different sizes of leaves to lay on. Leaves from potted dock plants were used in this experiment. The leaves were all approximately the same age, but were of different sizes. "Large" leaves were chosen to be approximately two to three times the size of "small" leaves.

Females were enclosed individually in cages made of fine mesh netting containing a small soil-filled pot into which each leaf was inserted. One group was given alternatively large leaves for three days, small leaves for three days and again large leaves for three days etc. for a period of 30 days. A second group of females was given only large leaves, the leaves being changed every three days. A third group was given only small leaves, the leaves again being changed every three days. The numbers of eggs laid in each clutch of each female were counted . No significant relationships were found between the size of leaves presented to females and either the numbers of eggs laid per clutch or the total numbers of clutches laid during the period of the experiment.

This result indicated that if females do alter the number of eggs they lay in response to the food supply that will be available to their offspring when they hatch, they do not do so at the level of the single leaf on which each clutch of eggs is laid. This result is perhaps not surprising, given that larvae are not confined to a single leaf after they have hatched and are therefore not solely dependent on it to supply the necessary food for the completion of their development.

Experiments were designed to investigate the possibility that females may choose to lay their eggs in positions which could minimize the risks of inter-clutch cannibalism. In an experiment in which females were housed individually in transparent plastic boxes provided with two cut dock leaves of similar size and age, there was some indication that females will lay first on one leaf, then on the other, then on the first one again and so on. It was therefore decided to repeat the experiment, giving females the choice of two living leaves instead of cut leaves.

This was attempted first of all by inserting one leaf of a potted dock plant into either end of a cage made of a metal frame covered with fine mesh netting. However, it was found that the cage was too large. Once a female had encountered one leaf, the other leaf was too far away to represent a "choice" of oviposition site. The experiment was repeated using shoe-boxes covered with netting rather than metal-frame cages. Although females could easily encounter both leaves, the leaves were often overlapping each other and did not represent very successfully the relative positions of two leaves that might be found on a normal plant. It was therefore decided to carry out the experiment using two leaves on single plants.

Potted dock plants, from which all but two leaves had been removed, were enclosed in net bags. The position in which females lay their eggs in relation to clutches they have previously laid was investigated by enclosing single females on each dock plant. The females were allowed to lay several successive clutches . The positions and time of laying of these clutches were recorded twice daily. No evidence was found to suggest that females show any preference for laying their eggs either on leaves where they had previously laid eggs or on egg-free leaves.

The same experiment was repeated to investigate whether or not females show any preference for laying their eggs either on a leaf which is egg-free or on a leaf which already has a clutch of another female. One female beetle was placed on each plant and when she had laid a clutch of eggs, she was replaced by a second female. The leaf on which each female had laid her clutch of eggs was noted. In half of the cases, the second female laid her clutch on the same leaf as the clutch of the first beetle; in the remaining cases, she laid her eggs on the other leaf. Since no preference was found, these experiments were not pursued any further.

An experiment was carried out to investigate any possible relationships between the order in which eggs are laid in a clutch and the order in which they hatch out, as this could have important implications for intra-clutch cannibalism since first-hatched larvae are more likely to be cannibals and later-hatching eggs are more likely to be victims. A female beetle was placed on each of 20 potted dock plants. Each plant was checked continuously in rotation for signs of females beginning to lay eggs. As soon as a female was spotted laying eggs, the time of laying of each egg was noted and at the same time a diagram was made of the position of each egg so that eggs could later be identified at hatching.

This in itself provided some interesting observations. The resulting clutch is roughly ovoid in shape, and appears to be composed of eggs laid in fairly regular lines. However, females do not lay eggs consecutively along regular lines. Instead, after laying the first egg, laying is alternated first to the right and then to the left of the original egg. This alternation is not always regular, but it results in most of the eggs not being laid adjacent to either an egg laid immediately previously or an egg laid immediately afterwards.

At the expected time of hatching, clutches of eggs were watched closely and as soon as a clutch of eggs began to hatch, the time of hatching of each egg was recorded. In this way, regression analysis could be carried out on the relationship between the order in which an egg is laid and the order in which it hatches. It was found that the relationship was statistically significant (Hatchorder = 11.5 + 0.145 Layorder. F=5.75, d.f.= 1,185, $0.01<p<0.025$, r.sq.= 3.0%). However, it was decided that a great deal of significance should not be attached to these results for several reasons. Firstly, it was difficult to come across a female that had just started to lay. Therefore relatively few clutches were observed and many of the laying patterns of clutches were incomplete. Likewise, it was difficult to watch continuously for the hatching of clutches and so the hatching of some clutches was missed altogether, or only partially observed. This reduced the eventual sample size even further. Secondly, it was difficult to know how much disturbance was being caused to the females by this particular method of observation. Also, observations often had to be made from an awkward position since eggs are laid on the undersides of leaves, perhaps reducing the accuracy of these observations. And thirdly, the r.sq. value is very low, indicating that there is a lot of variance in the results. Nevertheless the results are suficiently interesting to be worthwhile pursuing. A more efficient and accurate way of observing females laying eggs could perhaps be achieved by confining females to lay on pieces of leaf in petri dishes. It would be helpful to have more than one person making observations since a female laying her eggs was often missed because observations had already begun on another female, thus making the process of gathering data very time-consuming.

While investigating the nutritional benefits of cannibalism, a preliminary experiment was carried out to determine the effects of different diets on the survival, developmental rates and attained adult weights of larvae. The experiment was the same as the one described in Chapter 5, except that larvae were weighed every day on the Cahn electrobalance. But it was realized that this method caused too much disturbance which was detrimental to the development of the larvae and resulted in very few larvae reaching the adult stage. It was therefore decided to repeat the experiment using the method described in Chapter 6, where eggs were weighed before hatching and larvae were not weighed at all. Only the resulting weights of adults were recorded.

In connection with the experiments to investigate the possible involvement of chemical defence in cannibalism, I visited the laboratory of Professor J. Pasteels in Brussels. There I learned the technique of "milking" beetles to obtain their defensive secretion. I was also shown how to perform column chromatography on this secretion to obtain pure isoxazolinone glucoside compounds for use in the quantitative analysis of these compounds in beetle eggs. Subsequently, I spent several weeks practicing the various techniques I would need to carry out the analyses of defensive compounds in the eggs. However, I was unable, in the end to carry this out as the beetles I had in culture all died.

Development of the main areas of research

In recent years cannibalism has become recognized as being an extremely common and widespread phenomenon throughout the animal kingdom. It was therefore interesting, but perhaps not altogether surprising, to find that the larvae of *Gastrophysa viridula* eat the eggs of their own species. It can hardly be claimed, either, that the observed extent of cannibalism in this species was cause for great consternation. On average, only 4.1% of eggs within clutches were cannibalized by other clutch members. This represents less than 2 eggs of an average-sized clutch of 41 eggs. With roughly only 2% of the eggs in clutches being non-viable, this does not really qualify cannibalism in *G. viridula* for classification as a case of females laying infertile trophic eggs as early food provisioning for their young; particularly so, since not all non-viable eggs were unfertilized.

Nevertheless, two aspects of the cannibalism in this species proved to be sufficiently interesting to merit further investigation. If it had not been for these two particular aspects, it is quite possible that cannibalism in *Gastrophysa viridula*, as in so many other species, might well have been dismissed as being "unimportant" or "of little consequence".

The first point of interest is that larvae were found to be much more voracious cannibals when given an unlimited supply of young conspecific eggs than could ever have been deduced from the observations of cannibalism in normally-hatching clutches. The second point is that potentially viable eggs as well as non-viable eggs are eaten from within clutches. Most of the thesis is centred around inter-related problems arising from these two findings:- Do cannibals benefit in terms of individual fitness from eating conspecific eggs? If so, have females evolved any mechanisms to protect their eggs against inter-clutch cannibalism? Could individual fitness benefits be sufficient for cannibalism of even very close relatives to be advantageous in terms of inclusive fitness? - and , if this is the case, why is it that within-clutch cannibalism is of such limited extent?

Individual fitness benefits of cannibalism

The possible individual fitness benefits to be gained from being cannibalistic were investigated by raising larvae on different diets of dock leaves, conspecific eggs or combinations of both. Although larvae fed only on eggs did not survive to reach the second instar, it was not clear whether this was due to the eggs being an inadequate source of nutrition, or whether the solitary larvae were not stimulated to feed as they normally would when surrounded by other larvae. This point needs to be clarified by repeating the experiment, rearing the larvae in groups rather than singly.

Larvae fed on both dock leaves and conspecific eggs developed more quickly and had a higher probability of survival than non-cannibals fed only on dock leaves. This was true at least for larvae that, in addition to dock leaves, fed only on very young eggs during their first instar. Even if confirmation was obtained that eating eggs on their own is not sufficient to support larval development, the possibility still remains that eggs may contain some essential component of nutrition, such as protein, that is only found in small quantities in dock leaves.

Fitness benefits in terms of the resultant size of cannibalistic adults as compared to the size gained by non-cannibals were not detected, but the low survival rates in two of the treatment groups meant that the sample sizes were quite small. Although adult body size has been found to be correlated with both longevity and measures of fecundity in many species of insects and other invertebrates (see Charnov *et al.*, 1981), this was not found to be the case in *Gastrophysa viridula*. So even if cannibalistic larvae were found to result in larger adults than non-cannibals when larger sample sizes were obtained, this would not necessarily imply that cannibals had any fitness advantages in terms of longevity or fecundity over non-cannibals. However, this really needs to be tested directly on larvae raised either cannibalistically or non-cannibalistically.

Possible protective mechanisms against inter-clutch cannibalism

Since it has been shown that larvae gain fitness benefits in terms of developmental rates and survival and that larvae will readily eat young eggs given the opportunity, it would not be surprising to find that mechanisms to protect eggs against cannibalism had evolved. This idea is reinforced by the fact that the potential for inter-clutch cannibalism is apparently quite high. Second and third instar larvae range quite widely across many of the leaves on a dock plant and so are quite likely to encounter clutches of unhatched eggs. Female beetles able to protect their eggs against cannibalism by unrelated larvae would therefore be at a selective advantatage over females that did not protect their eggs.

Two inter-related ways in which females could protect their eggs against cannibalism emerged during the study. The first involves the position in which females lay their eggs in relation to previously laid clutches. In comparison to eggs laid at a distance of 0.5 cm. from from a previously laid clutch, it was found that eggs laid at a distance of only 1 cm. were afforded quite a high degree of protection against cannibalism by larvae hatching from the older eggs. The time taken to reach the introduced eggs was important. The mean time taken for larvae to reach eggs at a distance of 1 cm. was more than 12 times as great as the time taken for larvae to reach eggs 0.5 cm. away. Such a striking difference can probably be explained by the characteristic way in which larvae move after they have hatched. At first, they spread outwards in all directions from the centre of the area on which they hatched. It is likely that eggs only 0.5 cm. away from the edge of a clutch will lie within this initial expansion area and so will be certain to be encountered. However, after the initial expansion has taken place, the larvae move away, as a group, from the area on which they hatched and the chances of eggs further away from this area being encountered are much smaller. Depending on the directions taken by the larvae, some eggs may not be encountered at all before either the eggs hatch or the larvae move off the leaf.

One aspect of the "positional effect" is therefore the decreasing likelihood of eggs being encountered the further away they are from previously laid clutches. But this is not the only form of protection associated with this mechanism. Of the eggs that were encountered at either of the two distances from previously laid clutches, the more distant ones suffered less cannibalism. The most likely explanation for this was provided by the results from an experiment which showed that eggs develop an intrinsic protective mechanism against cannibalism which becomes increasingly effective with age. The more distant eggs would have had more time to develop this intrinsic protective mechanism than the eggs laid closer to the previously laid clutch before the hatched larvae reached them.

If the time costs of finding a leaf with no previously laid eggs on them are quite high, the best strategy for females might be to lay their eggs at a "safe" distance on the same leaf as previously laid eggs, even at the possible risk of losing a small number of eggs. If females are unable to tell the age of previously laid eggs, the "safe" distance would be expected to be that which would give optimal protection if the eggs in the previously laid clutch were on the point of hatching. Some experimental evidence was obtained to suggest that females do lay their eggs at relatively safe distances from previously laid clutches. But much more extensive field work is required to establish what exactly constitutes a "safe" distance in different circumstances and whether females do in fact conform to such safety standards!

Although the position in which females lay their eggs may be very important in protecting them against cannibalism by first instar larvae, such tactics are unlikely to offer much protection against the more widely-ranging second and third instar larvae. However, the intrinsic protective mechanism of eggs that plays a part in the positional effect may also protect eggs against cannibalism from older larvae to some extent. It was found that the amounts of eggs eaten in terms of volume and the actual numbers of eggs attacked by larvae declined with the age of the eggs. Even 2-day old eggs had some degree of intrinsic protection against cannibalism. It is therefore likely that there is only a very short period during which eggs are vulnerable to large losses through cannibalism, even from second and third instar larvae.

The nature of the intrinsic protective mechanism is an interesting problem that has not yet been fully explored. Defensive chemicals effective against potential egg predators such as ants have been found in the eggs of *G. viridula* (Pasteels *et al.*, 1986). Some indications to suggest that chemical defence may also be involved in the protection of eggs against cannibalism were found during the present study. Oleic acid is known to be effective in deterring the feeding behaviour of ants (Howard *et al.*, 1982). Pasteels *et al.* (1986) found oleic acid in the eggs of *G. viridula*, but only in trace amounts. However, they did not state the age of the eggs that they sampled and they only measured the quantities of free oleic acid present in the eggs. Fatty acids in glyceride form have also been shown to be effective deterrents of predacious ants (Iltis and Zweig, 1962 and Hinton, 1968). And in an experiment carried out as part of this thesis it was found that the relative concentration of total oleic acid (free oleic acid plus oleic acid incorporated in other forms) increases with age in *G. viridula* eggs. The relative concentrations of all other major fatty acids either declined or did not change with increasing age of the eggs.

These observations, together with the finding that there is a negative relationship between the susceptibility of eggs to cannibalism and their age, suggest that oleic acid may be involved in the protection of eggs not only against predation from other species, but also against intra-specific predation. However, direct confirmation is required that the absolute concentration of total oleic acid increases with the age of the eggs and also that oleic acid is efffective in deterring the cannibalistic habits of *G. viridula* larvae at the concentrations and in the forms in which it is found in older eggs.

Pasteels *et al.* (1986) found that an isoxazolinone glucoside compound present in the eggs of *G. viridula* was highly deterrent at natural concentrations to the feeding behaviour of ants. It would be interesting to check if this chemical is also effective in the protection of eggs against cannibalism.

Oleic acid and the isoxazolinone glucoside compound were found in the fluid contents of the eggs of *G. viridula*, but not on the exterior, in the gluey secretion covering the eggs (Pasteels *et al.*, 1986). The position of these chemicals indicates that they could only be effective in deterring predators or cannibals once at least one egg in a clutch has been punctured. In the experiment in which it was discovered that the susceptibility of *G. viridula* eggs to cannibalism declines with age, even in the trials with the oldest eggs in which cannibalism was greatly reduced, a small number of viable eggs were eaten. This finding is consistent with the idea that the intrinsic protective mechanism of the eggs is based on defensive chemicals present in the interior of the eggs.

If eggs do become more toxic with age, this could explain the otherwise anomalous finding that larvae fed on dock leaves plus conspecific eggs throughout all three larval instars did less well in terms of survival than larvae fed on dock leaves throughout all instars, but conspecific eggs only during their first instar. Eggs given during the larvae's first instar were changed daily whereas eggs given to larvae during their second and third instars were left for longer before replacing them with young eggs. The main difference between the two treatment groups was therefore that larvae given eggs during their first instar ate only very young eggs, whereas those receiving eggs throughout all three instars also ate some eggs that were more than two days old. This suggests that the older eggs may have a detrimental effect on the fitness of larvae. It would be very interesting to investigate more directly the possibility that such an effect is linked with the development of toxic defensive chemicals in ageing eggs.

If there is any substance in this possibility, it would then be worthwhile looking more closely at the effect that eating older eggs has on the future cannibalistic tendencies of larvae. Although, in an experiment, larvae were found to eat eggs when presented to them in either their first, second or third instars, these larvae had not had any previous experience of eating older eggs. It would be interesting to find out if such an experience could deter larvae from attempting to eat any eggs which they subsequently came across. If this were the case, one consequence might be that the level of inter-clutch cannibalism could be reduced below the level that might otherwise have been expected to prevail if larvae remained cannibalistic throughout all three instars, irrespective of whether or not they had eaten any older eggs.

The eggs that females lay may therefore be protected against inter-clutch cannibalism both by the position in which they are laid and by the intrinsic protective mechanism of the eggs themselves. It would therefore be very interesting in the future to investigate inter-clutch cannibalism in a field situation, paying particular attention, in the light of these findings, to the possible factors that may influence its extent.

Inclusive fitness and the limitation of within-clutch cannibalism

The limiting factor to the extent of within-clutch cannibalism is not the number of non-viable eggs present in clutches since it was found that some viable eggs are also eaten. Eating viable eggs within clutches would not be too surprising an occurrence if it could be shown that the gain in fitness to the cannibal is sufficient to outweigh the genetic loss of killing a related victim, according to Hamilton's (1964a) criterion for the selection of selfish behaviour. It has already been mentioned that there are individual fitness advantages to being cannibalistic on young eggs. To judge whether or not these advantages would be sufficient to satisfy Hamilton's criterion it was necessary to know the degree of relatedness between the eggs of a clutch.

This was tackled through an investigation of sperm competiton in *G. viridula*. It was found that the mechanism of sperm competition in this species is likely to be either one of sperm removal or repositioning with sperm mixing, or sperm mixing alone but where the amount of sperm transferred by males to females diminishes with the time of mating. Further experimental observations are required to determine the precise mechanism of sperm competition since the data obtained during the experiment was consistent with mathematical models of both of these alternative mechanisms.

However, the results were sufficient to confirm that the average degree of relatedness between the eggs of a particular clutch is likely to lie somewhere between 0.25 and 0.5, since clutches may consist of full- and half-siblings. The benefits in terms of survival gained by cannibals of young eggs in the laboratory experiment were certainly great enough to fulfill Hamilton's criterion, even if the larvae were to eat only full-siblings. So it would appear that it is not the insufficiency of personal fitness benefits to compensate for the costliness, in genetic terms, of eating siblings that has resulted in low-level within-clutch cannibalism - although this should only be taken as a tentative conclusion since confirmation is required that the benefits of cannibalism found in the laboratory are also sufficient in natural situations to fulfill Hamilton's criterion.

In a proximate sense, the reasons why within-clutch cannibalism should be so limited are fairly clear. The eggs in a clutch do not hatch out entirely synchronously. This can result in some eggs remaining unhatched and therefore vulnerable to cannibalism when the earliest hatched larvae have left their egg shells and are moving around within the clutch. However, newly hatched larvae do not immediately leave their egg shells and so, since the degree of asynchrony of hatching was found to be positively related to the clutch size, smaller clutches may result in complete hatching succes of viable eggs. It was found that clutches containing up to 38 eggs may result in complete hatching success of viable eggs. In clutches with more eggs than this "shoulder" value, a negative relationship was found between the percentage hatching success of viable eggs and the clutch size. Females laid a mean of 41 eggs per clutch, a number very close to the shoulder value and with the consequence that, as already stated, very few viable eggs were normally eaten within clutches. In 14 out of 23 cases, (61%), no viable eggs were eaten at all. And overall, a mean of less than 1% of viable eggs in clutches were eaten by other clutch members.

The question of why, in an evolutionary sense, females should lay clutch sizes that result in very limited within-clutch cannibalism is not quite so clear. Although it was found that eating young eggs, even if closely related, could be selectively advantageous, the situation would be very different if eating older eggs was detrimental rather than beneficial to the fitness of larvae. As discussed earlier, this might be the case if the intrinsic protective mechanism of eggs is based on the concentrations of toxic chemicals increasing with age in the eggs. Hamilton's criterion for the selection of selfish behaviour would no longer be fulfilled for within-clutch cannibalism of viable eggs on the point of hatching. A complicated situation might exist whereby females, having invested in chemical defence of their eggs to reduce the risks of inter-clutch cannibalism would consequently be selected to prevent their own offspring from eating any of these older, toxic eggs through within-clutch cannibalism. Larvae that had eaten viable eggs within clutches might subsequently not have such good prospects of surviving to adulthood as non-cannibalistic larvae or larvae that had eaten only non-viable eggs.

But if eating viable eggs within clutches is selectively disadvantageous, the question remains as to why any of these eggs should be eaten at all within clutches. Given that larvae cannot be deterred from eating eggs until at least one has been punctured, it may be that females are unable to completely eliminate within-clutch cannibalism of viable eggs without laying clutch sizes considerably below the shoulder value because of anatomical or physiological constraints on their mechanism of oviposition. It was found that most females lay a very distinctive sequentially alternating series of larger and smaller clutches throughout most of their egg-laying lives. Oviposition resulting in such a pattern precludes the possibility of females always laying clutch sizes of a particular value. In Chapter 2 the possibility of a "shoulder" clutch size was discussed. If such a shoulder size is found to exist, and If there are reasons why laying clutch sizes smaller than the shoulder value would be disadvantageous, such as the increased time costs of searching for oviposition sites or increased risks of parasitism and predation, the best compromise might be for females to lay, on average, clutch sizes close to the shoulder value, even at the risk of sometimes having some of her offspring eat viable eggs from within their clutches.

Kin recognition

It was found that first instar *Gastrophysa viridula* larvae made no distinction between related and non-related young eggs as victims of cannibalism. Although neither older larvae nor older eggs were included in the experiment, some of the foregoing ideas suggest reasons why a kin recognition system might not be expected in this species.

A kin recognition system could be advantageous if it paid, in terms of inclusive fitness, to eat non-relatives, but not to eat close relatives. However, if there is any substance in the indications that eating older eggs may be detrimental rather than beneficial to the fitness of larvae, it would not pay larvae to eat any older eggs, related or unrelated. Although larvae do eat some older eggs, this occurrence is apparently kept to a minimum. There is very little opportunity for larvae to eat older, viable eggs within their own clutches and it was shown that larvae will refrain from eating older eggs in a clutch once at least one of the eggs has been punctured.

A kin recognition system could still be advantageous if larvae commonly came across young eggs and if it was again advantageous to eat non-relatives, but not to eat close relatives such as siblings. However, the chances of larvae encountering sibling eggs that are still young may be quite low. And in any case, laboratory findings indicated that eating young eggs can result in sufficient personal benefits to make eating even full-siblings advantageous. If this finding holds true under natural conditions, a kin recognition system would not be beneficial.

Factors that may have influenced selection for an optimal clutch size in G. viridula

This thesis has not directly addressed the question of what factors may have influenced the evolution of clutch size in *Gastrophysa viridula*. However, the work on cannibalism has indirectly led to the consideration of possibilities which may be worthy of future investigations in this respect.

A first point to consider might be the reason why *G. viridula* females lay clutches at all, rather than laying eggs singly. By laying eggs singly, females would remove all risks of losing any of their eggs through intra-clutch cannibalism. However, there may be benefits from laying eggs in clutches which outweigh the small risk of losing a small number of eggs through intra-clutch cannibalism.

For example, Stamp (1980) and Chew and Robbins (1983) considered the possibility that laying eggs in clutches rather than singly may be beneficial in limiting parasitism and predation. For example, wasps may preferentially parasitize the outer eggs in clutches of Panamanian *Chlosyne lasinia*, thus protecting the inner eggs from attack (Chew and Robbins 1983).

Stamp (1980) and Chew and Robbins (1983) also discussed the possibility that laying eggs in clutches could be beneficial in enhancing the effects of aposematic colouration (such as exists in *G. viridula* eggs) in deterring predators from eating toxic eggs. Although they suggest that toxicity of eggs and conspicuous colouration would most likely have to have arisen independently; once evolved, laying eggs in clutches would become advantageous because the tasting of a toxic egg would deter a predator from eating other sibling eggs from within the same clutch.

This idea was originally discussed by Fisher (1930) for the case of gregariousness in larvae. Evidence presented by Wade and Breden (1986) appears to confirm the benefits of clutch size in connection with toxicity. They studied natural populations of a chrysomelid beetle, *Plagiodera versicolora* which feeds mainly on the leaves of willow trees of the genus *Salix.* The larvae secrete a defensive substance from eversible glands on the dorsolateral surface of the pronotum when disturbed. This behaviour is effective in repelling insect predators, and the effectiveness is a function of group size (Raupp, 1982). Wade and Breden (1986) found that the size of a group of larvae upon hatching was positively and significantly correlated with the mean duration of larval life. The larvae of *Gastrophysa viridula* also have dorsolateral glands from which a defensive fluid is secreted (Pasteels *et al.*, 1986). It is possible that the same advantages of clutch size apply in this species and would be worth investigating.

A third idea discussed by Stamp (1980) and Chew and Robbins (1983) is that gregarious larvae feed more efficiently in groups. Long (1953, 1955) found that larvae of *Pieris brassicae* reared in groups developed faster than larvae reared singly. However, Chew and Robbins (1983) pointed out that as feeding in groups evolves, behavioural adaptations for this way of feeding are also likely to evolve. It would therefore not be surprising to find that normally gregarious larve feed more efficiently in groups than singly, but this cannot be used as an argument for the advantages of laying eggs in clutches.

If there are advantages to laying eggs in clutches rather than singly, there may be a clutch size which maximizes the fitness of a female on a per clutch basis - the "Lack" clutch size (e.g. Parker and Courtney, 1984; Charnov and Skinner, 1985). The "Lack" clutch size could be calculated by determining the the clutch size that maximizes the product of the number of larvae surviving to adulthood per clutch and a measure of their mean fitness. An appropriate measure of fitness might be the lifetime egg production of female offspring (Skinner, 1985). The "Lack" clutch size will be affected not only by the benefits of laying eggs in clutches, but also by any detriments which may be associated with the clutch-laying habit.

In Chapter 2, section3, possible factors which may result in decreased numbers of surviving adult offspring and/or decreased fitness of the resulting offspring with increasing clutch size were discussed; namely, predation and parasitism, competition among larvae and intra-clutch cannibalism. Although in *G. viridula* the effects of predation and parasitism may be decreased with increasing clutch size for the reasons discussed above, it might also be the case that predation increases with increasing clutch size - for example, if larger clutches are more obvious to predators which are not affected by the toxicity of the eggs. (The case of syrphid larvae may be relevant here. When a syrphid egg is laid in the midst of a clutch of *G. viridula* eggs, the entire clutch is doomed to be eaten when the syrphid hatches (Whittaker *et al.* 1979)). It would be interesting to investigate the effects of predation on clutches of differing sizes under field conditions.

Similarly, the effects of intra-clutch cannibalism on the fitness of the resulting larvae from clutches could possibly be either positive or negative. In Chapter 2, section 3, the possible detrimental effects of larvae eating viable, possibly toxic eggs, on the fitness of larvae was discussed. However, it is also possible that Alexander's "ice-box hypothesis" may operate (Alexander, 1974, quoted in Polis, 1984). In other words, larvae gain nutritional benefits from eating eggs from within their own clutches. Evidence presented in Chapter 5 suggest that the former possibility is more likely. Nevertheless controlled experiments are required to conclusively distiguish between these two possibilities.

Determining the "Lack" clutch size for *Gastrophysa viridula* could provide some insight into the factors which have influenced the evolution of clutch size in this species. However, the "Lack" clutch size is the clutch size that maximizes a female's fitness on a per clutch basis, but natural selection will be expected to favour females that maximize their fitness over their entire lifetime (Parker and Courtney, 1984; Skinner, 1985).

It is commonly found that in birds (Charnov and Skinner, 1984) and insects (Charnov and Skinner, 1984; Skinner, 1985; Waage and Godfray, 1985) females lay clutch sizes that are on average smaller than the predicted "Lack" clutch size. Several suggestions have been put forward to explain this. One possibility is that by laying larger clutches, females suffer a higher risk of mortality, perhaps due to greater risks of predation during oviposition than when feeding or moving between oviposition sites (Weis *et al.*, 1983; Iwasa *et al.*, 1984). In the case of *Gastrophysa viridula*, there is no reason to believe that females are under greater risk of predation while laying eggs than when feeding. In both cases, the beetles remain at a particular position on a leaf for several minutes - (the time taken to lay a clutch of eggs was measured in 14 of the 23 clutches observed in this experiment and was 17.95 ± 2.10 minutes (n = 14). It has also been suggested that by laying larger clutches , females may suffer a reduced longevity independently of the risks of predation. This has been reported to be the case in many hymenoptera (Skinner, 1985), but experimental observations are required to investigate this possibility in *G. viridula*.

The rate at which surviving offspring are produced becomes important when considering the clutch size that will maximize a females' fitness over their entire lifetime and Parker and Courtney (1984) and Skinner (1985) have developed models to explore the implications of gain rate maximization. Parker and Courtney (1984) concluded that if females must spend a high proportion of their time in feeding to mature their eggs, their clutches will be expected to be smaller than the Lack clutch size. Green dock leaf beetles lay just less than one clutch of eggs per day on average (a mean of 36.73 ± 1.54 eggs per day) during their egg-laying period in laboratory conditions - see Chapter 5. After each clutch is laid they must feed in order to mature the next one. They could therefore incur quite high time costs in this way and the optimal clutch size would be predicted to be lower than the "Lack" clutch size.

On the other hand, both Parker and Courtney (1984) and Skinner (1985) predicted that the optimal clutch size would increase with increasing search costs for oviposition sites although it could never pay to lay more than the "Lack" clutch size. The search costs in this species are in fact likely to be quite small. One problem for females is the risk of having their eggs cannibalized by larvae from other clutches. They would be expected to lay their eggs in a position giving the best chance of minimizing this risk. By laying eggs close to a previously laid clutch of eggs females run the risk of having their eggs eaten by the earlier hatching larvae; but by laying their eggs only a short distance away on the same leaf, this risk is significantly reduced (see Chapter 6). They are not obliged to move very great distances, therefore, to find a "safe" place to lay their eggs in this respect.

They will certainly move between the leaves of a particular dock plant during their lifetime and possibly between different plants within a clump, but are unlikely to move between clumps of dock plants (Smith and Whittaker, 1980a). The search costs may be quite low in this species and a clutch size lower than the "Lack" clutch size may therefore be predicted to have been selected for.

There are quite a range of possible factors that might influence selection for an optimal clutch size in female insects. Experiments and observations, especially on natural populations, are required before the relative contributions of these factors acting on the determination of clutch size in *Gastrophysa viridula* can be elucidated.

Having discussed the ideas and contributions of present-day biologists to our understanding of clutch size problems, it is quite amazing to realize that many of the basic considerations underlying our approach to these problems had already been thought out over a century ago by Darwin (1871; also quoted in Charnov and Skinner, 1984).

> "Thus the fertility of each species will tend to increase, from the more fertile pairs producing a large number of offspring, and these from their mere number will have the best chance of surviving, and will transmit their tendency to greater fertility. The only check to a continued augmentation of fertility in each organism seems to be either the expenditure of more power and the greater risks run by the parents that produce a more numerous progeny, the contingency of very numerous eggs and young being produced of smaller size, or less vigorous, or subsequently not so well nurtured.

> To strike a balance in any case between the disadvantages which follow from the production of a numerous progeny, and the advantages (such as the escape of at least some individuals from various dangers) is quite beyond our powers of judgement."

More than one hundred years later, here we are, still struggling to try to weigh up these various advantages and disadvantages, and to find the balance between them.

Concluding remarks

One of the most valuable things that I have gained from carrying out the work for this thesis has been the gradual realization of how important cannibalism can be in influencing many features of an animal's life history. At the outset, what attracted me to studying cannibalism in the green dock leaf beetle was simply that it was something new and unexpected - at least for me. I did not think twice about making it the central theme of my research. It is perhaps just as well that at that stage I did not know very much about cannibalism in general, otherwise I might have been a bit more cautious about carrying on with something that could have seemed like an unpromising area on which to concentrate three years of research. After all, it is now realized that there is nothing unusual about cannibalism in the animal kingdom and in this particular species it is not even very spectacular in its extent.

But these are exactly the two aspects of cannibalism that make the study of this behaviour so interesting. The fact that cannibalism has evolved in so many species suggests that eating conspecifics can often be selectively advantageous. If this is the case, then the question that Dawkins asked in "The Selfish Gene" (1976, p. 89) - "Why is cannibalism relatively rare?" - is no longer appropriate. But a more relevant question might be - why is cannibalism so limited in extent in species such as *Gastrophysa viridula* ?

It seems quite possible that the very reason why cannibalism is so common across many species is also the reason why it may be very limited in extent within some species. If it is very advantageous to eat conspecifics, natural selection, in turn, may well have favoured mechanisms to avoid such predation. An observed limited extent of cannibalism within a species would then be the result, even though the potential for cannibalism might superficially have seemed much greater.

This leads on to a very interesting possibility. Many aspects of the behaviour, physiology or morphology of animals have generally been thought of as having evolved as a means of protection against inter-specific predation. However, since cannibalism is potentially an important form of predation in many species, it seems quite possible that many of these traits could equally, or even specifically, have evolved as a means of protection against intra-specific predation.

In this respect, some of the findings of this thesis suggest a potentially exciting area for future research - the investigation of the role played by chemical defence against cannibalism in species where it has previously been assumed to function primarily as a protective mechanism against predation from other species. More generally, it would be interesting to investigate any possible mechanisms that may have evolved to reduce the risks of cannibalism. There are a few scattered references in published papers suggesting where such mechanisms may exist. For example, Crump (1983) suggested that the extra-thick jelly capsules surrounding the eggs of some species of amphibians may deter cannibalism and Hurlbert *et al.* (1972) considered that the out-growths of the body wall of the predacious rotifer *Asplanchna* may have evolved specifically as anti-cannibalism devices. But there are no in-depth studies of these possibilities and I am sure that it is an area in which there is a great deal of scope for some very interesting future research.

REFERENCES

Alexander, R. D. 1974. The Evolution of Social Behavior. Annual Review of Ecology and Systematics, 5 : 325-383.

Andrewartha, H. G. 1971. The concept of Local Population and the Mechanisms of Negative Feedback in Natural Populations. pp. 189-198 in den Boer, P. J. and Gradwell, G. R. Dynamics of Populations. Wageningen, Pudoc.

Anon. 1980. Asulox Technical Bulletin. May and Baker. London.

Allais, J. P., Bergerard, J., Etienne, J. and Polonovski, J. 1964. Nature et Evolution des Lipides au Course de l'Embryogenese de *Locusta migratoria migratoides.* Journal of Insect Physiology, 10 : 753-772.

Backus, V. L. and Cade W. H. 1986. Sperm Competition in the field Cricket, *Gryllus integer.* Florida Entomologist, 69 : 722-728.

Banks, C. J. 1956. Observations on the Behaviour and Mortality in Coccinellidae before Dispersal from the Egg Shells. Proceedings of the Royal Entomological Society of London (A), 31 : 56-60.

Basedow, T. 1982. Untersuchungen zur Populationsdynamik des Siebenpunktmarienkafers *Coccinella septempunctata* L. (Col. : Coccinellidae) auf Getreidefeldern in Schleswig-Holstein von 1976-1978. Zeitschrift fuer Angewandte Entomologie, 93-94 : 66-82.

Bar, D and Gerling, D. 1985. Cannibalism in *Chrysopa carnea* (Stephens) (Neuroptera, Chrysopidae). Israel Journal of Entomology, 19 : 13-22.

Baur, B. 1984. Shell Size and Growth Rate Differences for Alpine Populations of *Arianta arbustorum* (L.) (Pulmonata: Helicidae). Revue Suisse de Zoologie, 91 : 37-46.

Baur, B. 1986. The Influence of Cannibalistic Egg Eating on the Growth of Young *Arianta arbustorum* (L.) (Helicidae). Snail Farming Research, 1: 9-17.

Baur, B. 1987. Can Cannibalistic Hatchlings of the Land Snail *Arianta arbustorum* Distinguish Between Sib and Non-Sib Eggs? Behaviour, 103 : 259-265.

Baur, B. and Baur, A. 1986. Proximate Factors Influencing Egg Cannibalism in the Land Snail *Arianta arbustorum* (Pulmonata, Helicidae). Oecologia (Berlin), 70 : 283-287.

Beaver, R. 1974. Intraspecific Competition Among Bark Beetle Larvae (Coleoptera : Scolytidae). Journal of Animal Ecology, 43 : 460-470.

Begon, M. and Parker, G. A. 1986. Should Egg Size and Clutch Size Decrease with Age? Oikos, 47 : 293-302.

Bentley, S. and Whittaker, J. B. 1979. Effects of Grazing by a Chrysomelid Beetle, *Gastrophysa viridula* on Competition between *Rumex obtusifolius* and *Rumex crispus*. Journal of Ecology, 67 : 79-90.

Bentley, S., Whittaker, J. B. and Malloch, A. J. C. 1980. Field Experiments on the Effects of Grazing by a Chrysomelid Beetle (*Gastrophysa viridula*) on Seed Production and Quality in *Rumex obtusifolius* and *R. crispus*. Journal of Ecology, 68 : 671-674.

Black, T. H. 1983. The Preparation and Reactions of Diazomethane. Aldrichimica Acta. 16 : 3-10.

Blaustein, A. R., Bekoff, M. and Daniels, T. J. 1987a. Kin Recognition in Vertebrates (Excluding Primates) : Empirical Evidence. pp. 287-331 in Fletcher, D. J. C. and Michener, C. D. (eds.). Kin Recognition in Animals. John Wiley and Sons. New York.

Blaustein, A. R., Bekoff, M. and Daniels, T. J. 1987b. Kin Recognition in Vertebrates (Excludung Primates) : Mechanisms, Functions and Future Research. pp. 333-357 in Fletcher, D. J. C. and Michener, C. D. (eds.). Kin Recognition in Animals. John Wiley and Sons. New York.

Blaustein, A. R. and O'Hara, R. K. 1982. Kin Recognition in *Rana cascadae* Tadpoles : Maternal and Paternal Effects. Animal Behaviour, 30 : 1151-1157.

Blum, M. S. 1970. The Chemical Basis of Insect Sociality. pp. 61-94 in M. Beroza (ed.). Chemicals Controlling Insect Behavior. Academic Press. New York.

Bobisud, L. E. 1976. Cannibalism as an Evolutionary Strategy. Bulletin of Mathematical Biology, 38 : 359-368.

Boorman, E. and Parker, G. A. 1976. Sperm (Ejaculate) Competition in *Drosophila melanogaster* and the Reproductive Value of Females to Males in Relation to Female Age and Mating Status. Ecological Entomology, 1 : 145-155.

Boyce, J. M. 1946. The Influence of Fecundity and Egg Mortality on the Population Growth of *Tribolium confusum* Duval. Ecology, 27 : 290-302.

Brower, J. H. 1975. Sperm Precedence in the Indian Meal Moth, *Plodia interpunctella.* Annals of the Entomological Society of America, 68 : 78-80.

Brower, L. P. 1961. Experimental Analyses of Egg Cannibalism in the Monarch and Queen Butterflies, *Danaus plexippus* and *D. gillippus* berenice. Physiological Zoology, 34 : 287-296.

Brown, H. D. 1972. The Behaviour of Newly Hatched Coccinellid Larvae (Coleoptera : Coccinellidae). Journal of the Entomological Society of South Africa, 35 : 149-157.

Canard, M. 1970. L'oophagie des Larves du Premier Stade de Chrysope (Neuroptera, Chrysopidae). Entomologia Experimentalis et Applicata, 13 : 21-36.

Carrick, R. 1938. The Life-history and Development of *Agriolimax agrestis,* the Grey Field Slug. Transactions of the Royal Society of Edinburgh, 59 : 563-597.

Cavers, P. B. and Harper, J. C. 1958. *Rumex obtusifolius* L. and *R.crispus* L. Library Chronicle, (Edition. 11), No. 1686. List of British Vascular Plants, No. 325, 12 : 737-766.

Chapman, R. N. 1928. Quantitative Analysis of Environmental Factors. Ecology, 9 :111-122.

Chapman, R. N. 1933. The Causes of Fluctuations of Populations of Insects. Proceedings of the Hawaiin Entomological Society, 8 : 279-297.

Charnov, E. L., Los-Denhartogh, R. L., Jones, W. T. and Van Den Assem, J. 1981. Sex Ratio Evolution in a Variable Environment. Nature, 289 : 27-33.

Charnov, E. L. and Skinner, S. W. 1984. Evolution of Host Selection and Clutch Size in Parasitoid Wasps. The Florida Entomologist, 67 : 5-21.

Charnov, E. L. and Skinner, S. W. 1985. Complementary Approaches to the Understanding of Parasitoid Oviposition Decisions. Environmental Entomology, 14 : 383-391.

Chevalier, J. R. 1973. Cannibalism as a Factor in First Year Survival of Walleye in Oneida Lake. TRansactions of the American Fisheries Society, 102 : 739-744.

Chew, F. S. and Robbins, R. K. 1983. Egg laying in Butterflies, in The Biology of Butterflies, Symposia of the Royal Entomological Society of London, 11 : 65-79.

Choudhuri, D. and Bagh, R. 1974. On the Sub-social Behaviour and Cannibalism in *Schizodactylus monstrosus* (Orthoptera : Schizodactylidae). Revue d'Ecologie et de Biologie du Sol, 11 : 569-573.

Cideciyan, M. A. and Malloch, A. J. C. 1982. Effects of Seed Size on the Germination , Growth and Competitive Ability of *Rumex crispus* and *Rumex obtusifolius*. Journal of Ecology, 70 : 227-232.

Cochran, D. G. 1979. A Genetic Determination of Insemination Frequency and Sperm Precedence in the German Cockroach. Entomologia Experimentalis et Applicata, 26 : 259-266.

Coleman, C. S. and Frauenthal, J. C. 1983. Satiable Egg Eating Predators. Mathematical Biosciences, 63 : 99-119.

Colinvaux, P. A. 1973. Introduction to Ecology. Wiley. New York.

Corbet, P. S. and Griffiths, A. 1963. Observation on the Aquatic Stages of Two Species of *Toxorhynchites* (Diptera : Culicidae) in Uganda. Proceedings of the Royal Entomological Society of London. (A), 32 : 125-135.

Coyne, J. 1968. *Laspeyresia ingens*, a Seedworm Infesting Cones of Longleaf Pine. Annals of the Entomological Society of America, 61 : 1116-1122.

Croft, B. A. and McMurty, J. A. 1972. Comparative Studies of Four Strains of *Typhlodromus occidentalis* Nesbitt (Acarina : Phytoseiidae) IV. Life History Studies. Acarologia, 12 : 460-470.

Crombie, A. C. 1944. On Intraspecific and Interspecific Competition in Larvae of Graminivorous Insects. Journal of Experimental Biology, 20 : 135-151.

Crump, M. L. 1983. Opportunistic Cannibalism by Amphibian Larvae in Temporary Aquatic Environments. The American Naturalist, 121 : 281-287.

Daloze, D., Braekman, J. C. and Pasteels, J. M. 1986. A Toxic Dipeptide from the Defence Glands of the Colorada Beetle. Science, 233 : 221-223.

Daloze, D. and Pasteels, J. M. 1979. Production of Cardiac Glycosides by Chrysomelid Beetles and Larvae. Journal of Chemical Ecology, 5:63-77.

Daly, P. J. and Ryan, M. F. 1983. Density-related Mortality of the Flour Beetle, *Tribolium confusum* Duval. Research in Population Ecology, 25 : 210-219.

Darwin, C. 1871. The Descent of Man and Selection in Relation to Sex. John Murray. London.

Davey, K. G. 1985. The Female Reproductive Tract. pp. 1-14 in Kerkut, G.A., Gilbert, L.I. (eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology. 1. Embryogenesis and Reproduction. Pergamon Press, Oxford.

Dawkins, R. 1976. The Selfish Gene. Oxford University Press. New York.

DeAngelis, D. L., Cox, D. and Coutant, C. 1979. Cannibalism and Size Dispersal in Young-of-the-Year Largemouth Bass : Experiment and Model. Ecological Modelling, 8 : 133-148.

Deroe, C. and Pasteels, J. M. 1982. Distribution of Adult Defence Glands in Chrysomelids (Coleoptera : Chrysomelidae) and its Significance in the Evolution of Defence Mechanisms within the Family. Journal of Chemical Ecology, 8 : 67-82.

Desharnais, R. A. and Costantino, R. F. 1980. Genetic Analysis of a Population of *Tribolium.* VII. Stability : Response to Genetic and Demographic Perturbations. Canadian journal of Genetics and Cytology, 22 : 577-589.

Desharnais, R. A. and Liu, L. 1987. Stable Demographic Limit cycles in Laboratory Populations of *Tribolium castaneum.* journal of Animal Ecology, 56 : 885-906.

Dickinson, J. L. 1986. Prolonged Mating in the Milkweed Leaf Beetle (*Labidomera clivicollis clivicollis,* Coleoptera : Chrysomelidae) : aTest of the Sperm - Loading Hypothesis. Behavioral Ecology and Sociobiology, 18 : 331-338.

Dickinson, J. L. 1988. Determinants of Paternity in the Milkweed Leaf Beetle. Behavioral Ecology and Sociobiology, 23 : 9-19.

Diekmann, O., Nisbet, R. M., Gurney, W. S. C. and van den Bosch, F. 1986. Simple Mathematical Models for Cannibalism : A Critique and a New Approach. Mathematical Biosciences, 78 : 21-46.

Dimetry, N. Z. 1974. The Consequences of Egg Cannibalism in *Adalia bipunctata* (Coleoptera: Coccinellidae). Entomophaga, 19: 445-451.

Dixon, A. F. G. 1959. An Experimental Study of the Searching Behaviour of the Predatory Coccinellid Beetle *Adalia decempunctata* (L.). Journal of Animal Ecology, 28 : 259-281.

Duelli, P. 1981. Larval Cannibalism in Lacewings (Neuroptera : Chrysopidae). Research in Population Ecology, 23 : 49-67.

Eckert, A. W. 1974. The Owls of North America. Garden City, New York. Doubleday.

Eibl-Eibesfelt, I. 1961. The Fighting Behavior of Animals. Scientific American, 205 : 112-121.

Eickwort. K. R. 1971. The Ecology of *Labidomera clivicollis,* a Relatively Rare Milkweed Beetle Closely Related to the Colorado Potato Beetle (Coleoptera: Chrysomelidae, Doryphorini). Ph. D. dissertation, Cornell University. 123 pp.

Eickwort, K. R. 1973. Cannibalism and Kin Selection in *Labidomera clivicollis* (Coleoptera : Chrysomelidae). The American Naturalist,107 : 452-453.

Elbadry, E. A. and Fleschner, C. A. 1965. The Feeding Habits of Adults of *Chrysopa california* Coquillett. (Neuroptera: Chrysopidae). Bulletin de la Societe Entomologique d'Egypte, 49 : 359-366.

Eliot, J. M. 1973. The diel Activity pattern, Drifting and Food of the Leech *Erpobdella octoculata* (L.) (Hirundinea : Erpobdellidae) in a Lake District Stream. Journal of Animal Ecology, 42 : 449-459.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Oxford University Press. Oxford.

Fisher, R. C. 1970. Aspects of the Physiology of Endoparasitic Hymenoptera. Biological Reviews, 46 : 243-278.

Fitch, J. E. 1964. A Relatively Unexploited Population of Pismo Clams, *Tivela stultorum* (Mawe,1823) (Veneridae). Proceedings of the Malacological Society of London, 36 : 309-312.

Fletcher , D. J. C. and Blum, M. S. 1983. Regulation of Queen Numbers By Workers in Colonies of Social Insects. Science, 219 : 312-314.

Force, D. C. 1966. Reactions of the Green Dock Beetle, *Gastrophysa cyanea* (Coleoptera : Chrysomelidae) to its Host and certain Non-host Plants. Annals of the Entomological Society of America, 59 : 1119-1125.

Forney, J. 1976. Year Class Formation in the Walleye (*Stizostedion vitreum vitreum*) Population of Oneida Lake, New York, U.S.A. 1966-1973. Journal of the Fisheries Research Board Of Canada, 33 : 783-792.

Fox, L. R. 1975. Cannibalism in Natural Populations. Annual Review of Ecology and Systematics, 6 : 87-106.

Frauenthal, J. C. 1983. Some Simple Models of Cannibalism. Mathematical Biosciences, 63 : 87-98.

Getz, W. M., Bruckner, D. and Parisian, T. R. 1982. Kin Structure and the Swarming Behavior of the Honey Bee *Apis mellifera.* Behavioral Ecology and Sociobiology, 10 : 265-270.

Gilbert, L. I. 1967. Changes in Lipid Content During the Reproductive Cycle of *Leucophaea maderae* and Effects of the Juvenile Hormone on Lipid Metabolism in vitro. Comparative Biochemistry and Physiology, 21 : 237-257.

Gilbert, N. and Gutierrez, A. P. 1973. A Plant-Aphid-Parasite Relationship. Journal of Animal Ecology, 42 : 323-340.

Godfray, H. C. J. 1986. Models for Clutch Size and Sex Ratio with Sibling Interaction. Theoretical Population Biology, 30 : 215-231.

Godfray, H. C. J. 1987. The Evolution of Clutch Size in Parasitic Wasps. The American Naturalist, 129 : 221-233.

Gould, F., Holtzmann, G., Rabb, R. and Smith, M. 1980. Genetic Variation in Predatory and Cannibalistic Tendencies of *Heliothis virescens* strains. Annals of the Entomological Society of America, 73 : 243-250.

Gunter, G. 1947. Observations on Breeding of the Marine Catfish, *Galeichthys felis* (Linneaus). Copeia : 217-223.

Gurtin, M. E. and Levine, D. S. 1979. On Predator - Prey Interactions with Predation Dependent on Age of Prey. Mathematical Biosciences, 47 : 207-219.

Gwynne, D. T. 1984. Male Mating Effort, Confidence of Paternity, and Insect Sperm Competition. pp 117-149. in Smith, R.L. (ed.). Sperm Competition and the Evolution of Animal Mating Systems. Academic Press. New York.

Hamilton, W. D. 1963. The Evolution of Altruistic Behavior. The American Naturalist, 97 : 354-356.

Hamilton, W. D. 1964a. The Genetical Evolution of Social Behaviour. I. Journal of Theoretical Biology, 7 : 1-16.

Hamilton, W. D. 1964b. The Genetical Evolution of Social Behaviour. II. Journal of Theoretical Biology, 7 : 17-52.

Hamilton, W. D. 1987. Discriminating Nepotism : Expectable, Common, Overlooked. pp.417-437 in D. J. C. Fletcher and C. D. Michener (eds.) Kin Recognition in Animals. John Wiley and Sons. New York.

Haskins, C. P. 1970. Researches in the Biology and Social Behaviour of Primitive Ants., in L. R. Aronson, E. Tobach, D. S. Lehrman and J. S. Rosenblatt (eds.). Development and Evolution of Behavior. W. H. Freeman. San Francisco.

Hastings, A. 1987. Cycles in Cannibalistic Egg - Larval Interactions. Journal of Mathematical Biology, 24 : 651-666.

Hastings, A. and Costantino, R. F. 1987. Cannibalistic Egg - Larval Interactions in *Tribolium* : An Explanation for the Oscillations in Population Numbers. The American Naturalist, 130 : 36-52.

Hawkes, O. A. M. 1920. Observations on the Life-History, Biology and Genetics of the Ladybird Beetle, *Adalia bipunctata* (Mulsant). Proceedings of the Zoological Society of London, 1920 : 475-490.

Hinton, H. E. 1968. Structure and Protective Devices of the Egg of the Mosquito *Culex pipiens*. Journal of Insect Phisiology, 14 : 145-161.

Ho, F. K. and Dawson, P. S. 1966. Egg Cannibalism by *Tribolium* larvae. Ecology, 47 : 318-322.

Holcik, J. 1977. Changes in Fish Community of Kikava Reservoir with Particular Reference to Eurasian Perch. Journal of the Fisheries Board of Canada, 34 : 1734-1747.

Holloway, J. K. 1964. Projects in Biological Control of Weeds. pp. 650-670 in P. Deback and E. I. Schlinger (eds.). Biological Control of Insects, Pests and Weeds. Chapman and Hall. London.

Holmes, W. G. and Sherman, P. W. 1982. Kin Recognition in Animals. American Scientist, 71 : 46-55.

Hoogland, J. L. 1985. Infanticide in Prairie Dogs: Lactating Females Kill Offspring of Close Kin. Science, 230: 1037-1040.

Howard, D. F., Blum, M. S., Jones, T. H. and Phillips, D. W. 1982. Defensive Adaptations of Eggs and Adults of *Gastrophysa cyanea* (Coleoptera : Chrysomelidae). Journal of Chemical Ecology, 8 : 453-462.

Howard, D. F. and Tschinkel, W. R. 1976. Aspects of Necrophoric Behavior in the Red Imported Fire Ant, *Solenopsis invicta*. Behaviour, 56 : 157-179.

Hrdy, S. 1979. Infanticide Among Animals: A Review, Classification, and Examinationof the Implications for Reproductive Strategies of Females. Ethology and Sociobiology, 1 : 13-40.

Hurlbert, S. H., Mulla, M. S. and Willson, H. R. 1972. Effects of an Organophosphorus Insecticide on the Phytoplankton, Zooplankton, and Insect Populations of Fresh-Water Ponds. Ecological Monographs, 42 : 269-299.

Iltis, W. G. and Zweig, G. 1962. Surfactant in Apical Drop of Eggs of some Culicine Mosquitoes. Annals of the Entomological Society of America, 55 : 409-415.

Ito, Y., Tsubaki, Y. and Osada, M. 1982. Why do *Luehdorfia* Butterflies Lay Eggs in Clusters? Researches on Population Ecology, 24 : 375-387.

Iwasa, Y., Suzuki, Y. and Matsuda, H. 1984. Theory of Oviposition Strategy of Parasitoids. Theoretical Population Biology, 26 : 205-227.

Johnson, R. G. 1959. Spatial Distribution of *Phoronopsis viridis* Hilton. Science, 129, 1221.

Jonasson, P. 1971. Population Studies on *Chironomus anthracinus*. pp. 220-231 in Den Boer, P. J. and Gradwell, G. (eds.). Dynamics of Populations. Wageningen, Pudoc.

Jones, J. S. 1982. Of Cannibals and Kin. Nature, 299 : 202-203.

Kaddou, I. K. 1960. The Feeding Behavior of *Hippodamia quinquesignata* (Kirby) Larvae. University of California Publications in Entomology, 16 : 181-232.

Kehat, M. 1968. The Feeding Behaviour of *Pharoscymnus numidicus* (Coccinellidae), Predator of the Date Palm Scale *Parlatoria blanchardi*. Entomologia Experimentalis et Applicata, 11 : 30-42.

Klausnitzer, B. and Forster, G. 1971. Przyczynek do Znajomosci Morfologii jaj Kiliku Strodkowo-Europejskich Gatunkow Chrzaszczy z Rodziny Chrysomelidae (Coleoptera). Polskie Pismo Entomologiczne Seria B Entomologia Stoswana, 41 : 429-437.

Klomp, H. and Teerink, B. J. 1967. The Significance of Oviposition Rates in the Egg Parasite, *Trichogramma embryophagum* Htg. Archives Neerlandaises de Zoologie, 17 : 350-375.

Kruuk, H. 1972. The Spotted Hyena. Chicago, Illinois. University of Chicago Press.

Lack, D. 1947. The Significance of Clutch Size. Parts I and II. Ibis, 89 : 302-352.

Lack, D. 1954. The Natural Regulation of Animal Numbers. Clarendon Press. Oxford.

Lawton, J. H. 1970. Feeding and Food Energy Assimilation in Larvae of the Damselfly *Pyrrhosoma nymphula* (Sulz.) (Odonata: Zygoptera). Journal of Animal Ecology, 39 : 669-689.

Letsinger, J. T. and Gromko, M. H. 1985. The Role of Sperm Numbers in Sperm Competition and Female Remating in *Drosophila melanogaster.* Genetica, 66 : 195-202.

Levine, D. S. 1981. On the Stability of a Predator - Prey System with Egg-eating Predators. Mathematical Biosciences, 56 : 27-46.

Liebman, E. 1933. Some Observations on the Breeding Habits of Palestine Cichlidae. Proceedings of the Zoological Society of London, 85-87.

Linsenmair, K. E. and Linsenmair, C. 1971. Paarbildung und Paarzusammenhalt bei der Monogamen Wusstenassel *Hemilepistus reaumuri* (Crustacea, Isopoda, Oniscoidea). Zeitschrift fur Tierpsychologie, 29 : 134-155.

Linsenmair, K. E. 1972. Die Bedeutung Familienspezifischer "Abzeichen" fur den Familienzusammenhalt bei der Sozialen Wustenassel *Hemilepistus reaumuri* Audouin u. Savigny (Crustacea, Isopoda,Oniscoidea). Zeitschrift fur Tierpsychologie, 31 : 131-162.

Lipsitz, E. Y. and McFarlane, J. E. 1970. Total Lipid and Phospholipid During the Life Cycle of the House Cricket, *Acheta domesticus* (L.). Comparative Biochemistry and Physiology, 34 : 699-705.

Lipsitz, E. Y. and McFarlane, J. E. 1971. Analysis of Lipid During the Life Cycle of the House Cricket, *Acheta domesticus.* Insect Biochemistry, 1: 446-460.

Liu, L. and Cohen, J. E. 1987. Equilibrium and Local Stability in a Logistic Matrix Model for Age-structured Populations. Journal of Mathematical Biology, 25 : 73-88.

Loekle, D. M., Madison, D. M. and Christian, J. J. 1982. Time Dependency and Kin Recognition of Cannibalistic Behavior Among Poeciliid Fishes. Behavioral and Neural Biology, 35 : 315-318.

Long, D. 1953. Effects of Population Density on Larvae of Lepidoptera. Transactions of the Royal Entomological Society of London, 104 : 543-585.

Long, D. 1955. Observations on Sub-Social Behaviour in Two Species of Lepidopterous Larvae, *Pieris brassicae* L. and *Plusia gamma* L. Transactions of the Royal Entomological Society of London, 106 : 421-436.

Lonsdale, D., Heinle, D. and Siegfried, C. 1979. Carnivorous Feeding Behavior of the Adult Calanoid Copepod *Acartia tonsa* Dana. Journal of Experimental Marine Biology and Ecology, 36 : 235-248.

Lorenz, K. 1966. On Aggression. Bantam Books. New York.

Marriner, T. F. 1926. The Two Spot Ladybird. Discovery, 1926 : 407-409.

Maynard Smith, J. and Price, G. R. 1973. The Logic of Animal Conflict. Nature, 246 : 15-18.

Meffe, G. K. and Crump, M. L. 1987. Possible Growth and Reproductive Benefits of Cannibalism in the Mosquitofish. The American Naturalist, 129 : 203-212.

Mertz, D. B. and Robertson, J. R. 1970. Some Developmental Consequences of Handling, Egg-Eating, and Population Density for Flour Beetle Larvae. Ecology, 51 : 989-998.

Michener, C. D. 1974. The Social Behavior of the Bees. Harvard University Press, Cambridge. Massachusettes.

Minitab Reference Manual (Minitab Release 5.1). NWD 44. 1986. University of Manchester Regional Computer Centre. Manchester.

Mitchell, R. 1975. The Evolution of Oviposition Tactics in the Bean Weevil, *Callosobruchus maculatus.* (F.). Ecology, 56 : 696-702.

Mrowka, W. 1984. Brood Care Motivation and Hunger in the Mouthbrooding Cichlid *Pseudocrenilabrus multicolor.* Behavioural Processes, 9 : 181-190.

Mrowka, W. 1986. Satiation Restores Brood Care Motivation in the Female Mouthbrooder *Pseudocrenilabrus multicolor* (Cichlidae). Physiology and Behavior, 38 : 153-156.

Mrowka, W. 1987. Filial Cannibalism and Reproductive Success in the Maternal Mouthbrooding Cichlid Fish *Pseudocrenilabrus multicolor.* Behavioral Ecology and Sociobiology, 21 : 257-265.

Nagai, Y., Nagai, S. and Nishikawa, T. 1971. The Nutritional Efficiency of Cannibalism and an Artificial Feed for the Growth of Tadpoles of the Japanese Toad (*Bufo vulgaris* sp.). Agricultural and Biological Chemistry, 35: 697-703.

Nakamura, K. and Ohgushi, T. 1981. Studies on the Population Dynamics of a Thistle-Feeding Lady Beetle, *Henosepilachna pustulosa* (Kono) in a Cool Temperate Climax Forest. II. Life Tables, Key-factor Analysis and Detection of Regulatory Mechanisms. Researches on Population Ecology, 23 : 210-231.

Niemerko, P., Wlodawer, P. and Wojtczak, A. F. 1956. Lipid and Phosphorous Metabolism During Growth of the Silkworm (*Bombyx mori*). Acta Biologiae Experimentalis, Vars. 17 : 255-276.

Nikoloskii, G. V. 1969. Theory of Fish Population Dynamics. Edinburgh, Oliver and Boyd.

O'Connor, R. 1978. Brood Reduction in Birds: Selection for Fratricide, Infanticide and Suicide. Animal Behaviour, 26 : 79-96.

Pajunen, V. I. 1983. Prey Selection by Larvae of *Potamonectes griseostriatus* (Degeer) (Coleoptera : Dytiscidae). Annuario Zoologico de Fennici, 20 : 31-35.

Park, T., Leslie, P. M. and Mertz, D. B. 1964. genetic Strain and Competition in Populations of *Tribolium* . Physiological Zoology, 37 : 97-160.

Parker, G. A. 1970a. Sperm Competition and its Evolutionary Consequences in the Insects. Biological Reviews, 45 : 525-567.

Parker, G. A. 1970b. Sperm Competition and its Evolutionary Effect on Copula Duration in the Fly *Scatophaga stercoraria*. Journal of Insect Physiology, 16 : 1301-1328.

Parker, G. A. and Begon, M. 1986. Optimal Egg Size and Clutch Size : Effects of Environmental and Maternal Phenotype. The American Naturalist, 128 : 573-592.

Parker, G. A. and Courtney, S. P. 1984. Models of Clutch Size in Insect Oviposition. Theoretical Population Biology, 26 : 27-48.

Parker, G. A. and Mock, D. W. 1987. Parent-Offspring Conflict over Clutch Size. Evolutionary Ecology, 1 : 161-174.

Parker, G. A. and Smith, J. L. 1975. Sperm Competition and the Evolution of the Precopulatory Passive Phase Behaviour in *Locusta migratoria migratorioides*. Journal of Entomology, 49 : 155-171.

Pasteels, J. M., Braekman, J. C., Daloze, D. and Ottinger, R. 1982. Chemical Defence in Chrysomelid Larvae and Adults. Tetrahedron, 38 : 1891-1897.

Pasteels, J. M., Daloze, D. and Rowell-Rahier, M. 1986. Chemical Defence in Chrysomelid Eggs and Neonate Larvae. Physiological Entomology, 11 : 29-37.

Pasteels, J. M., Rowell-Rahier, M., Braekman, J. C. and Daloze, D. 1984. Chemical Defences in Leaf Beetles and their Larvae: The Ecological, Evolutionary and Taxonomic Significance. Biochemical Systematics and Ecology, 12 : 395-406.

Pienkowski, R. L. 1965. The Incidence and Effect of Egg Cannibalism in First-Instar *Coleomegilla maculata lengi* (Coleoptera : Coccinellidae). Annals of the Entomological Society of America, 58 : 150-153.

Polis, G. A. 1980. The Effect of Cannibalism on the Demography and Activity of a Natural Population of Desert Scorpions. Behavioral Ecology and Sociobiology, 7 : 25-35.

Polis, G. A. 1981. The Evolution and Dynamics of Intraspecific Predation. Annual Review of Ecology and Systematics, 12 : 225-251.

Polis, G. A. 1984. Intraspecific Predation and "Infant Killing" Among Invertebrates. pp. 87-104 in Hausfater, G. and Hrdy, S. B. (eds.). Infanticide : Comparative and Evolutionary Perspectives. Aldine. New York.

Popova, O. A. and Sytina, L. A. 1977. Food and Feeding Relations in Eurasian Perch (*Perca fluviatilis*) and Pike Perch (*Stizostedion lucioperca*). Journal of the Fisheries Board of Canada, 34 1559-1570.

Potts, G. R. and Vickerman, C. D. 1974. Studies in the Cereal Ecosystem. Advances in Ecological Research, 8 : 107-197.

Pritchard, G. 1964. The Prey of Dragonfly Larvae in Ponds of Northern Alberta. Canadian Journal of Zoology, 42 : 785-800.

Quiring, D. T. 1985. Effect of Larval Cannibalism on the Development and Reproductive Performance of *Agromyza frontella* (Rondani) (Diptera: Agromyzidae). Annals of the Entomological Society of America, 78 : 429-432.

Radovich, J. 1962. Effects of Sardine Spawning Size and Environment on Year-class Production. California Fish and Game, 48 : 123-140.

Raupp, M. J. 1982. Spatial Distribution and Seasonal Abundance of the Imported Willow Leaf Beetle, *Plagiodera versicolora* (Laich.) : the Effects of Plant Nutrition and Defense, Physical Factors, and Activities of Competitors and Predators. Ph.D. dissertation. University of Maryland, College Park.

Reed, J. and Stenseth, N. C. 1984. Evolution of Cannibalism in an Age-structured Population. Bulletin of Mathematical Biology, 46 : 371-377.

Rich, E. 1956. Egg cannibalism and Fecundity in *Tribolium*. Ecolgy, 37 : 109-120.

Ricker, W. F. 1954. Stock and Recruitment. Journal of the fisheries Board of Canada, 11 : 559-623.

Riemann, J. G. and Thorson, B. J. 1974. Viability and Use of Sperm after Irradiation of the Large Milkweed Bug. Annals of the Entomological Society of America, 67 : 871-876.

Salt, G. 1961. Competition Among Insect Parasitoids. Symposia of the Society of Experimental Biology, 15 : 96-119.

Sakaluk, S. K. 1986. Sperm Competition and the Evolution of Nuptial Feeding Behaviour in the Cricket *Grylloides supplicans* (Walker). Evolution, 40 : 584-593.

Schlager, G. 1960. Sperm Precedence in the Fertilization of Eggs in *Tribolium castaneum.* Annals of the Entomological Society of America, 53 : 557-560.

Sempala, S. D. K. 1983. Interactions Between Immature *Aedes africanus* (Theobold) and Larvae of Two Predatory Species of *Toxorhinchites* (Diptera : Culicidae) in Zika Forest, Uganda. Bulletin of Entomological Research, 73 : 19-24.

Sherman, P. 1980. Reproductive Competition and Infanticide in Belding's Ground Squirrels and Other Animals. in R. D. Alexander and D. Tinkle (eds.). Natural Selection and Social Behavior: Recent Research and New Theory. Chiron. New York.

Shimizu, N. and Fujiyama, S. 1986. Aggressive Encounters between Males in *Chrysolina aurichalcea* (Coleoptera : Chrysomelidae). Journal of Ethology, 4 : 11-15.

Simmons, L. W. 1987. Sperm Competition as a Mechanism of Female Choice in the Field Cricket, *Gryllus bimaculatus.* Behavioral Ecology and Sociobiology, 21 : 197-202.

Siva-Jothy, M. T. 1984. Sperm Competition in the Libellulidae (Anisoptera) with Special Reference to *Crocothemis erythraea* (Brulle) and *Orthetrum cancellatum* (L.). Advances in Odonatology, 2 : 195-207.

Skinner, S. W. 1985. Clutch Size as an Optimal Foraging Problem for Insect Parasitoids. Behavioral Ecology and Sociobiology, 17 : 231-238.

Skurdal, J., Bleken, E. and Stenseth, N. C. 1985. Cannibalism in Whitefish (*Coregonus lavaretus*). Oecologia (Berlin), 67 : 566-571.

Smith, R. H. and Lessells, C. M. 1985. Oviposition, Ovicide and Larval Competition in Granivorous Insects. pp. 423-448 in R. M. Sibly and R. H. Smith, (eds.). Behavioural Ecology : Ecological Consequences of Adaptive Behaviour. Blackwells, Oxford.

Smith, R. W. 1977. The Dynamics of the Animal Community Associated with Broadleaved Dock (*Rumex obtusifolius.* L.). Unpublished Ph.D. thesis. University of Lancaster, 1987.

Smith, R. W. and Whittaker, J. B. 1980a. The Influence of Habitat Type on the Population Dynamics of *Gastrophysa viridula* Degeer (Coleoptera : chrysomelidae). Journal of Animal Ecology, 49 : 225-236.

Smith, R. W. and Whittaker, J. B. 1980b. Factors Affecting *Gastrophysa viridula* Populations (Coleoptera : Chrysomelidae) in Different Habitats. Journal of Animal Ecology, 49 : 537-548.

Sokoloff, A. 1974. The biology of *Tribolium*. Volume 2. Oxford University Press, Oxford.

Sonleitner, F. J. 1961. Factors Affecting Egg Cannibalism and Fecundity in Populations of Adult *Tribolium castaneum* Herbst. Physiology and Zoology, 34 : 233-255.

Sotherton, N. W., Wratten, S. D. and Vickerman, G. P. 1985. The Role of Egg Predation in the Population Dynamics of *Gastrophysa polygoni* (Coleoptera) in Cereal Fields. Oikos, 43 : 301-308.

Speight, R. I. and Whittaker, J. B. 1987. Interactions between the Chrysomelid Beetle, *Gastrophysa viridula*, the Weed *Rumex obtusifolius* and the Herbicide Asulam. Journal of Applied Ecology, 24 : 119-129.

SPSSX User's Guide. Edition 2. 1986. M^{c}Graw-Hill Book Company. London.

Stamp, N. E. 1980. Egg Deposition Patterns in Butterflies: Why do Some Species Cluster their Eggs Rather Than Deposit them Singly? American Naturalist, 115 : 367-380.

Stanley, J. 1938. The Egg-Producing Capacity of Populations of *Tribolium confusum* Duv. as Affected by Intensive Cannibalistic Egg-Consumption. Canadian Journal of Research, 16, Section D : 300-306.

Stenseth, N. C. 1985. On the Evolution of Cannibalism. Journal of Theoretical Biology, 115 : 161-177.

Stenseth , N. C. and Reed, J. 1978. A Comment on Bobisud's Paper on Evolution of Cannibalism. Bulletin of Mathematical Biology, 40 : 541-545.

Svoboda, J. A., Pepper, J. H., and Baker, J. L. 1966. On the Lipids of the Eggs of the Grasshopper Species *Aulocara eliotti*. Journal of Insect Physiology, 12 : 1549-1565.

Tepedino, V.J. and Frohlich, D. R. 1984. Fratricide in *Megachile rotundata*, a Non-social Megachilid Bee: Impartial Treatment of Sibs and Non-sibs. Behavioral Ecology and Sociobiology, 15 : 19-23.

Thompson, R. W., Di Biasio, D. and Mendes, C. 1982. Predator -Prey Interactions : Egg-eating Predators. Mathematical Biosciences, 60 : 109-120.

Tschinkel, W. 1978. Dispersal Behavior of the Larval Tenibrionid Beetle *zophobus rugipes*. Physiological Zoology, 51 : 300-313.

Ullyette, G. C. 1950. Competition for Food and Allied Phenomena in Sheep Blowfly Populations. Philosophical Transactions of the Royal Society of London, 234 : 77-174.

Volterra, V. 1931. Variations and Fluctuations of the Number of Individuals in Animal Species Living Together. pp. 409-448 in chapman, R. N. (ed.). Animal Ecology. New York. McGraw-Hill.

Vroman, H. E., Kaplanis, J. E. and Robbins, W. E. 1965. Effect of Allotectomy on Lipid Biosynthesis and Turnover in the Female American Cockroach, *Periplaneta americana*. Journal of Insect Physiology, 11 : 897- 904.

Waage, J. K. 1979a. Adaptive Significance of Postcopulatory Guarding of Mates and Nonmates by Male *Calopteryx maculata* (Odonata). Behavioral Ecology and Sociobiology, 6 : 147-154.

Waage, J. K. 1979b. Dual Function of the Damselfly Penis: Sperm Removal and Transfer. Science, 203 : 916-918.

Waage, J. K. 1984. Sperm Competition and the Evolution of Odonate Mating Systems. pp. 251-290 in R.L.Smith (ed.). Sperm competition and the Evolution of Animal Mating Systems. Academic Press. New York.

Waage, J. K. and Godfray, H. C. J. 1985. Reproductive Strategies and Population Ecology of Insect Parasitoids. pp 449-470 in R. M. Sibly and R. H. Smith, eds. Behavioural Ecology, Ecological Consequences of Adaptive Behaviour. Blackwells, Oxford.

Waage, J. K. and Ng, S. M. 1984. The Reproductive Strategy of a Parasitic Wasp. I. Optimal Progeny and Sex Allocation in *Trichogramma evanescens* . The Journal of Animal Ecology, 53 : 401-416.

Wade, M. J. 1980. An Experimental Study of Kin Selection. Evolution, 34 : 844-855.

Wade, M. J. and Breden, F. 1986. Life History of Natural Populations of the Imported Willow Leaf Beetle, *Plagiodera versicolora* (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America, 79 : 73-79.

Waldorf, E. 1971. Selective Egg Cannibalism in *Sinella curviseta* (Collembola : Entomobryidae). Ecology, 52 :673-675.

Walker, W. F. 1980. Sperm Utilization Strategies in Nonsocial Insects. The American Naturalist, 115 : 780-799.

Wang, R. and Kok, L. T. 1986. Life History of *Megacerus discoidus* (Coleoptera : Bruchidae), a Seedfeeder of Hedge Bindweed in Southern Virginia. Annals of the Entomological Society of America, 79 : 359-363.

Way, M. J. 1966. Significance of Self-Regulatory Dispersal in Control of Aphids by Natural Enemies. in I. Hodek (ed.). Ecology of Aphidophagous Insects. Academia, Prague.

Weiss, A. E., Price, P. W. and Lynch, M. 1983. Selective Pressures on Clutch Size in the Gall Maker *Arteomyia carbonifera.* Ecology, 64 : 688-695.

Welcomme, R. C. 1967. The Relationship between Fecundity and Fertility in the Mouthbrooding Cichlid Fish *Tilapia leucostica.* Journal of Zoology, 151 : 453-468.

White, E. G. and Huffaker, C. B. 1969. Regulatory Processes and Population Cyclicity in Laboratory Populations of *Anagasta kuhniella* (Zeller) (Lepidoptera : Phycitidae). I. Competition for Food and Predation. Researches on Population Ecolgy. Kyoto, 11: 57-83.

Whittaker, J. B. 1982. The Effect of Grazing by a Chrysomelid Beetle, *Gastrophysa viridula*, on Growth and survival of *Rumex crispus* on a shingle bank. Journal of Ecology, 70 : 291-296.

Whittaker, J. B., Ellistone, J. and Patrick, C. K. 1979. The Dynamics of a Chrysomelid Beetle, *Gastrophysa viridula*, in a Hazardous Natural Habitat. Journal of Animal Ecology, 48 : 973-986.

Wilson, E. O. 1971. The Insect Societies. Harvard University Press. Cambridge, Mass.

Wilson, E. O. 1987. Kin Recognition : An Introductory Synopsis. in Fletcher, D. J. C. and Michener, C. D. Kin Recognition in Animals (eds.). John Wiley and Sons. New York.

Wilson, E. O., Durlach, N. I. and Roth, L. M. 1958. Chemical Releasers of Necrophoric Behavior in Ants. Psyche, 65 : 154-161.

Woodhead, A. P. 1985. Sperm Mixing in the Cockroach *Diploptera punctata.* Evolution, 39 : 159-164.

Woodin, S. A. 1974. Polychaete Abundance Patterns in a Marine Soft-Sediment Environment : The Importance of Biological Interactions. Ecological Monographs, 44 :171-187.

Woodin, S. A. 1976. Adult - Larval Interactions of Dense Infaunal Assemblages : Patterns of Abundance. Journal of Marine Research, 34 : 25-41.

Yurkiewicz, W. J. and Oelsner, J. 1969. Neutral Lipid Metabolism During Embryonic Development of the Indian Meal Moth, *Plodia interpunctella* (Hubner). Comparative Biology and Physiology, 28 : 953-959.

Zimmering, S. and Fowler, G. N. 1966. X-Irradiation of the *Drosophila* male and its Effects on the Number of Sperm Transferred to the Female. Zeitschrift fuer Vererbungslehre, 98 : 150-151.

Zwoelfer, H. 1973. Possibilities and Limitations in the Biological Control of Weeds. European and Mediteranean Plant Protection Organization Bulletin, 3 : 19-30.