Larval competition and cannibalism in the Indian meal moth, *Plodia interpunctella*.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by Douglas James Reed.

July, 1998

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Abstract

The bulk of this thesis deals with various aspects of the biology of the Indian meal moth, *Plodia interpunctella* (Hübner). The moth is a widespread pest of stored food products, and has been used as a model system for studying population dynamics, both in Liverpool and elsewhere.

Chapter 1 describes the biology of the moth and its parasitoid, *Venturia canescens* (Gravenhorst), and general methods for rearing and handling them. There were considerable problems with a protozoan pathogen, *Mattesia dispora*, affecting the *P. interpunctella* cultures in the early years of this project, and these and other problems are also described in chapter 1.

Chapter 2 describes experiments to determine the effect of *P. interpunctella* cannibalism on the parasitoid, *V. canescens*. In individual encounters between parasitised and unparasitised *P. interpunctella* larvae, the parasitised larvae were more often cannibalised. A test to see whether this preferential cannibalism of parasitised larvae also occurred with different relative densities of parasitised and unparasitised larvae in food, failed due to the *Mattesia dispora* infection.

Chapter 3 used mathematical modelling to explore the possible effects of the differential cannibalism discovered in the previous chapter, and of host cannibalism in general, on the population dynamics of a host-parasitoid system. The general results were that host cannibalism was always more detrimental to the parasitoid than the host. These results have interesting implications for the evolution of cannibalism in host-parasitoid systems and for biological control strategies.

Chapters 4 and 5 are closely linked, and explore the effects of initial density on cohorts of *P. interpunctella* from eggs through to adults. Chapter 4 describes a factorial experiment to look at the effects of a wide range of larval densities on egg to adult survival and adult reproductive potential. Responses to density differed between males and females, but in general, higher densities led to smaller size and lower survival. Density affected reproductive investment through changes in body size. Chapter 5 followed cohorts through their life at 4 different densities. Two different stage-frequency analysis techniques were compared, and a method based on multiple regression was found to be the most appropriate. This analysis showed that the main density-dependent responses were in the later larval instars, with consequent effects on larval and pupal mortality and duration, and adult size and life span.

Chapter 6 explores an effect of adult age on offspring development rate found by a previous student at Liverpool. Through a series of experiments, I found that these "super-eggs" supposedly laid by older females were probably an artefact of experimental technique with contamination between different days' egg production. The effect disappeared in more tightly controlled experiments.

In chapter 7 I describe some incidental work on another parasitoid species, *Diadegma chrysostictos*, that I was hoping to use for the original aim of this project, which was to study one host – two parasitoid species population dynamics. I discovered that this species probably had a complementary sex determination system, which made it unsuitable for use in population cages.

The final chapter summarises and discusses my results in the light of attempts to understand the long-term population dynamics of *P. interpunctella* and its natural enemies.

Acknowledgements

Many people have helped me through my time at Liverpool, chief among them my ever patient and understanding supervisors, Mike Begon and Dave Thompson. Steve Sait collaborated with me on the final set of experiments, and his help and support was invaluable. Tom Heyes helped me with some of the practical work in my earlier experiments, and was able to find many obscure and useful items of equipment and supplies for me. Ian Harvey, Matt Gage, Roger Bowers and Nina Weddel have also given me valuable help and advice along the way. Mike Bonsall and Rob Butcher provided me with *Plodia interpunctella* cultures from Silwood Park and Dundee respectively. Dr. G. Marris gave me the *Diadegma chrysostictos* cultures, and Dr. M. Shaw confirmed their identification for me. Paul Ode provided me with wild-type and white-eye strains of *Bracon hebetor*. I have made it this far only because of the support of the people closest to me: my Mother and Father, Paul, Eric, Louise, Susanna and Harriet. Your intelligence and understanding have been an inspiration to me. Thank you.

When I arrived here almost 5 years ago, as well as my normal Ph.D. research, I began an in-depth study of laboratory animals. In the intervening time I have encountered many different and fascinating species. Many of these were discovered after publishing my first identification handbook[†] , and below I present a revised and updated checklist.

Honey-bran worm (*Larvae harvey*) — Extremely vocal, leaves behind a sticky trail.
Honey moth (Corcyra casey) — Stockpiles large quantities of honey.
Inventor fish (*Dungotron hutchinson*) — Constructs elaborate edifices of unknown purpose.
The Knell (*Troglodytes rob*) — Likes beer and caves, in roughly equal amounts.
Lopsided lizard (*Asymmetrica gage*) — Likes to spend the night with other males.
Migratory butterfly (*Philophila cook*) — Recently spotted in Scandinavia.
Music moth (*Mathematica lynch*) — Occasionally makes a deep and VERY loud noise.
Olive weevil (*Ferrobolus legros*) — Goes to great lengths to seek out other weevil species.
Purple catfish (*Piscophila shaw*) — Attracted to the colour purple.
Red-haired bat (*Rufocephala willson*) — Likes the company of other animals.
Seal dung fly (*Nic mao-dictator*) — Highly energetic dancing display.
Shark-finned earwig (*Forficula tomkins*) — Playful. Recently invaded Australia.

This may not be an exhaustive checklist, and I apologise for any omissions. Recently, a group of primate species (*Psycho* spp) moved into my study area, and while I haven't had time to get to know them fully, they seem harmless enough. In keeping with other primate studies I have given them silly names: "Russell", "Laura", "Paul", "Rob & Lil" (they go together as one), "Vera" and "Mary". All of these animals have proved enormously rewarding and stimulating to work with, and while I now intend to move on to study other ecosystems, I will always remember my time spent here with fondness.

[†] **Reed *et al*. (1993)** A handbook for the identification of laboratory animals. Fictitious Academic Books, Liverpool. 6pp

1. Introduction and general methods

1.1 Introduction

This thesis describes several different and rather loosely connected bodies of work which are hard to draw under one banner for the purpose of a general introduction. Each of the following chapters has its own introduction to set the scene for the work described there. Here, I will give a description of how the project as a whole developed, before describing my main study animals and the general techniques used.

The original aim of the project was to study the long-term population dynamics of a three-species, host-parasitoid-parasitoid system. Long-term population studies — usually based around the stored product moth, *Plodia interpunctella* — had become something of a speciality at Liverpool, and the host-parasitoid-parasitoid system was the next logical step to investigate. One of the parasitoids, *Venturia canescens*, was already in culture at Liverpool, and its behaviour in population cages was already well known. I had to locate a suitable second parasitoid species quickly, in order to give me enough remaining time to run it in population cages for at least 2 years.

The first parasitoid that I tried was *Diadegma chrysostictos*, which initially looked promising. It was an Ichneumonid, similar to *V. canescens* in size and general behaviour, yet it was a sexual species, while *V. canescens* was parthenogenetic. This provided the possibility of an interesting comparison of the two sexual strategies in a population dynamics context. However, I quickly discovered problems in culturing *D. chrysostictos*, and in **chapter 7** I describe evidence that this was caused by a complementary sex determination system previously unknown in this species. This made it completely unsuitable for use in population cages, so I had to try another species, *Bracon hebetor*. Once I had obtained cultures of *B. hebetor* and begun to rear them in quantity, it became clear that it would be very difficult to design a population cage that would allow a reasonable comparison of the competitive abilities of the two parasitoid species. *B. hebetor*'s small size and ability to burrow through the food medium in search of hosts meant that in the standard design of population cage used in the other studies it would have forced the host, and then itself, to extinction almost immediately. This realisation,

coupled with the sudden loss of my *B. hebetor* cultures to disease meant that I was going to have severe difficulty in setting up and running successful populations in the remaining time available to me.

By this time I had also done the first experiment described in **chapter 2**, which demonstrated that *P. interpunctella* larvae parasitised by *V. canescens* were more likely to be cannibalised than healthy larvae. Based on the success of this, and with the support of my supervisors, I abandoned the population studies in favour of pursuing the possibilities of cannibalism in *P. interpunctella*. However, shortly after I began this new line of research, the moth cultures that I was using became infected with a chronic protozoan pathogen, *Mattesia dispora*. The cultures were maintained and used by several people, and while we recognised that something was wrong and tried several times to re-establish healthy cultures, it was a year before the true nature of the infection (and thus the correct cure) became apparent. I set up several large-scale experiments to look at parasitism and host cannibalism at different densities during this period, and all of them failed due to the high pupal and adult mortality caused by the disease. I also did the theoretical work on modelling host cannibalism in host-parasitoid population dynamics, described in **chapter 3** during this time.

The cannibalism experiments that I had been planning required very large numbers of moth larvae to set up, yet it was taking a long time to build up culture levels after eradicating the disease. In the mean time, I decided to follow up a curious effect of adult age on the development rate of their offspring noted by a previous student working on *P. interpunctella* at Liverpool. She found that older moths appeared to lay "super-eggs", which developed faster. However, my experiments showed that this was probably an artefact of experimental technique rather than a real effect. This work is described in **chapter 6**.

Shortly after I finished this work, the cultures became reinfected with *M. dispora*, and were abandoned completely. I changed to using another culture of *P. interpunctella* that had been kept in another part of the building and had remained free of infection. After a long period of illness of my own, I collaborated with Dr. S. Sait in doing the final body of work described in **chapters 4 and 5**. This explored the effects of initial density on the development of larvae and adult reproductive potential in cohorts of *P. interpunctella*.

In the following sections I describe the main study animals that I used, and the general methods of culturing and handling.

1.2 *Plodia interpunctella*

1.2.1 Life history

Plodia interpunctella Hübner is an extremely widespread and cosmopolitan, Pyralid moth pest of stored foods and food products. Most commonly known as the Indian meal moth, other names include the meal worm moth, the compressed vegetable moth and the cloaked knothorn (Richards and Thomson, 1932). *P. interpunctella* probably originated in Asia, but global trading of the foodstuffs that it infests has allowed the moth to spread across the world. It now occurs on every continent except Antarctica, even reaching remote island groups such as Hawaii and the Virgin isles (Richards and Thomson, 1932).

The caterpillars are major pests of stored food products such as dried fruits, cereals and nuts (Cox and Bell, 1991), however they will also feed on an extremely wide variety of other substrates. Richards and Thompson (1932) review reports of *P. interpunctella* feeding on at least 86 different food types including fresh and dried fruit, seeds and nuts, grain, flour, chocolate, vegetables, rice, dried plants, sweets and cakes, *Cannabis* and old books!

The adult moths lay an average of 200 eggs over a period of about a week (Snyman, 1949; Silhacek and Miller, 1972; Podoler, 1974a). When these eggs hatch, the caterpillars burrow through the substrate, feeding and passing through five (and rarely six or seven) instars (Richards and Thomson, 1932; Cox and Bell, 1991). Once feeding is complete, the final instar caterpillar enters a wandering phase, moving up and out of the food to find a pupation site (Lindfield, 1990). The wandering stage caterpillar can diapause in response to low temperature or short photoperiods (Cox and Bell, 1991). Egg to adult development time varies with temperature: about 60 days at 20°C, 30 days at 25°C and 22 days at 30°C (Richards and Thomson, 1932; Silhacek and Miller, 1972; Cox and Bell, 1991). In tropical regions, *P. interpunctella* can pass through up to eight generations a year, but in temperate areas this is reduced to just one or two generations (Cox and Bell, 1991).

There were two different cultures of *P. interpunctella* maintained at Liverpool. The 25°C moths were taken from a laboratory culture in Dundee and the strain had been maintained in the laboratory for many years. They were used mainly for behavioural

experiments and for studies on the behaviour of *Venturia canescens*. The 28°C cultures came from a colony maintained at Imperial College, Silwood Park and had also been maintained as a laboratory strain for many years. At Liverpool, these had been used mainly for long-term population studies involving *V. canescens* and various pathogens. I started my work on the 25°C cultures, but disease and practical constraints forced me to use the 28°C cultures for later experiments.

1.2.2 Culturing methods: 25°C cultures

The animals for the 25°C cultures were reared on a 10:1:1 by volume mixture of wheat bran, yeast and glycerol respectively. This was mixed in 5L batches using a food mixer and stored in a freezer between uses. It later became necessary to heat sterilise the bran at 80°C before use to prevent the spread of disease (see below), but this did not appear to alter the food's consistency or it's palatability to the *P. interpunctella* larvae.

All of the culturing and experiments were carried out in controlled-temperature rooms at 25±2°C. Humidity was not controlled but was monitored and remained fairly constant at 60-70% RH, and there was a 16:8 hour light:dark cycle. Under these conditions the egg to adult development time was 30-35 days.

Eggs were collected daily from adult moths using an 'egg machine' (Figure 1.1) which was recharged with about 100 fresh moths every 3–4 days. The eggs were distributed into glass jars using a measure so that approximately 400 eggs were in each jar. The jars were then filled with 400 ml of food each and covered with a square of paper towel and a square of nylon gauze secured tightly with an elastic band.

1.2.3 Culturing methods: 28°C cultures

In my last set of experiments I switched to using the 28°C cultures. The culturing methods were broadly similar to those of the 25°C cultures, but used a different food recipe. The food was mixed in batches consisting of 2000g heat sterilised wheat bran, 400g yeast, 600ml glycerol, 600ml clear honey, 2.5g methyl paraben and 2.5g ascorbic acid. All experiments were carried out in incubators at 28°C, and a 15:9 hour light:dark cycle.

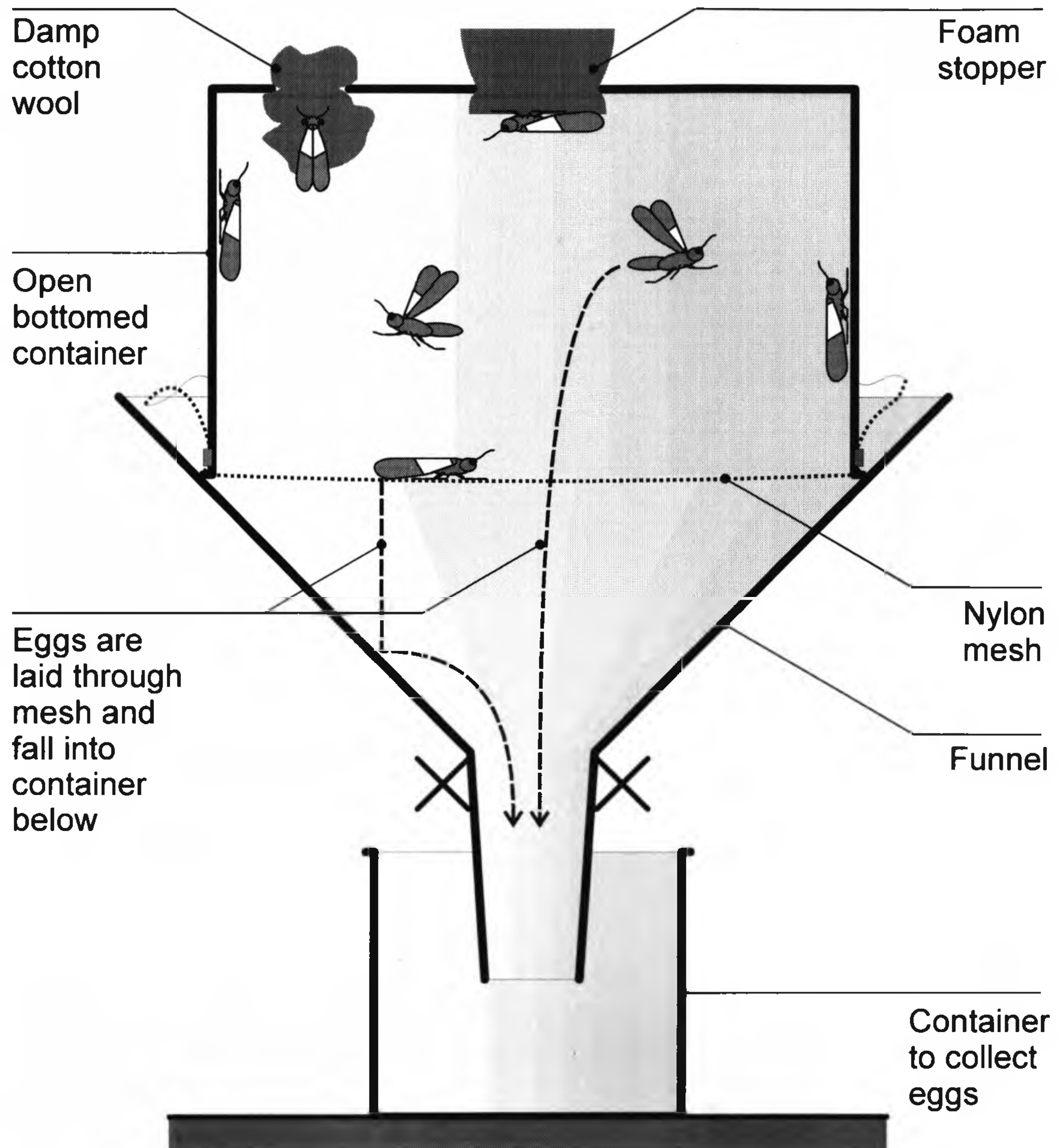

Figure 1.1 The 'egg machine' used to collect eggs from *P. interpunctella* adults.

1.2.4 Handling

Larvae for experiments were extracted from the food by emptying a culture jar into an experimental sieve with a mesh size of 1–2mm. The larvae were driven out of the food and through the sieve into the pan below by heat from a light bulb placed above the sieve. Larvae of the correct stage could then be found easily and removed from the sieve pan. The later instars (3–5) could be handled quite easily using soft, insect-handling forceps, while a fine brush was used for moving eggs and early instars (1 and 2). Adults were usually chilled in a refrigerator for an hour, and they were then docile enough to be handled with soft forceps.

Instar	Head Capsule Width (mm)
1st	0.15 – 0.20
2nd	0.28 – 0.33
3rd	0.40 – 0.45
4th	0.60 – 0.70
5th	0.85 – 1.15

Table 1.1 Head capsule widths for the instars of *P. interpunctella*. Taken from Lindfield (1990 Appendix IV).

Table 1.1 shows that there was no overlap in head capsule widths between instars, so it was easy to tell the instars apart visually (especially the later instars), without needing to measure each individual. For some experiments I distinguished between 'early' and 'late' stages of each instar: early individuals had a body that was narrower or the same width as the head capsule, while late individuals' bodies were wider than their head capsules. The head capsule is hardened and so can not expand between moults, whereas the rest of the body is very flexible and expands as the animal grows between moults. Thus the relative widths of the head and body give a rough indication of how close an individual is to moulting into the next instar. By using only early larvae in short-term (48 hour) experiments I could ensure that none of the larvae changed instar during the course of the experiment.

1.2.5 Egg Hatch

The hatch-rate of eggs was measured as a part of several experiments. The basic method was to transfer the eggs to the sticky side of an address label in a Petri dish using a fine brush, and then to leave the eggs in an incubator until they hatched. The glue on the label did not appear to affect the ability of the eggs to hatch, but it did help to prevent hatched larvae from moving around and cannibalising unhatched eggs. When the eggs on the label were examined under a microscope, hatched eggs were translucent, while unhatched eggs were yellowish and opaque.

1.2.6 Disease in the 25°C cultures

During the course of my experimental work, the 25°C cultures were infected by a protozoan pathogen, *Mattesia dispora*, probably from infected bran. Although we were aware of a problem in the cultures for many months and tried to re-establish clean stocks several times, the true nature of the disease was only identified about a year after the

original infection. All of the experiments that I set up during this time failed due to high disease mortality.

M. dispora is a neogregarine microsporidian that infects the fat bodies of lepidopteran caterpillars. In *P. interpunctella* this often causes the fat body cells to lyse, killing the larva or preventing it from pupating (Tanada and Kaya, 1993). Infected individuals that do manage to pupate and emerge as adults suffer much lower fertility and survival rates. The pathogen forms spores that can be transmitted horizontally in frass and by cannibalism, or vertically in the eggs. Once infected, it is hard to rid a culture of the disease, since the spores are very hardy, and have several transmission methods. Infected cultures are characterised by low survival and fertility rates and the appearance of large numbers of moribund, pale greenish wandering stage caterpillars that never pupate (Snyman, 1949). However, the only reliable method of identifying the pathogen is to look for the spores in stained samples of tissue (Poinar, 1984) from wandering stage caterpillars or adults (see below).

Once the nature of the infection was recognised, several steps were taken to try to rid the 25°C cultures of the disease and to prevent reinfection:

- All equipment was heat sterilised to > 80°C or soaked in strong disinfectant between uses.
- The wheat bran used in the food was heat sterilised to > 80°C before use.
- All culture jars were autoclaved and cleaned as soon as possible after moths had finished emerging in them.
- New, clean stocks were set up by collecting eggs from single pairs of moths which were subsequently killed and tested for *M. dispora* (see testing procedure below). Only eggs that came from uninfected parents were used to set up new cultures. Once a complete generation of moths had been set up in this way, the normal culturing system was resumed, but with regular testing of larvae for the presence of the disease.

The staining procedure for detecting *M. dispora* was as follows (Poinar, 1984):

1. Smear the tissue sample onto a slide and air dry.
2. Fix in methanol for 3–4 minutes.

3. Air dry.
4. Dilute 1 drop of Giemsa stain with 1 cc of distilled water.
5. Stain smear for 15 minutes.
6. Wash in distilled water.
7. Air dry.
8. Examine under a high powered (>100x) light microscope. The spores appear as clumps of thick walled lemon-shaped structures.

1.3 *Venturia canescens*

1.3.1 Life History

Venturia canescens Gravenhorst (see Figure 1.2) is an Ichneumonid parasitoid which attacks a variety of stored product pest lepidopteran species, including *P. interpunctella*, and is used for biological control of these pests (Press *et al.*, 1982; Press and Arbogast, 1991). Other generic names have included *Nemeritis*, *Idechthis*, *Exidechthis*, and *Devorgilla* (Corbet and Rotheram, 1965). It is a solitary endoparasitoid, so that only a single adult develops from each parasitised host. *V. canescens* is parthenogenetic — females lay unfertilised eggs that develop into fully fertile females — and males are very rarely seen (Richards and Thomson, 1932).

Adult wasps live for about 2 days in the absence of food, but can live for up to 40 days if fed on honey or glucose solution. During this time they can mature and lay up to 400 eggs. The adults search for suitable hosts by using chemical cues to home in on patches of hosts then by probing with their ovipositor (Corbet, 1971; Waage, 1978; Kuwahara *et al.*, 1983; Nemoto *et al.*, 1987). An egg is held in a special chamber at the end of the ovipositor, so that when the ovipositor pierces a suitable host the egg can be laid in less than a second. Once the egg is laid and the ovipositor is withdrawn from the host, a new egg has to be moved to the chamber using a special 'cocking' motion (Rogers, 1972). When the wasp inserts its ovipositor, it is able to detect whether the host has already been parasitised and choose whether or not to lay another egg (Rogers, 1972; Hubbard *et al.*, 1987).

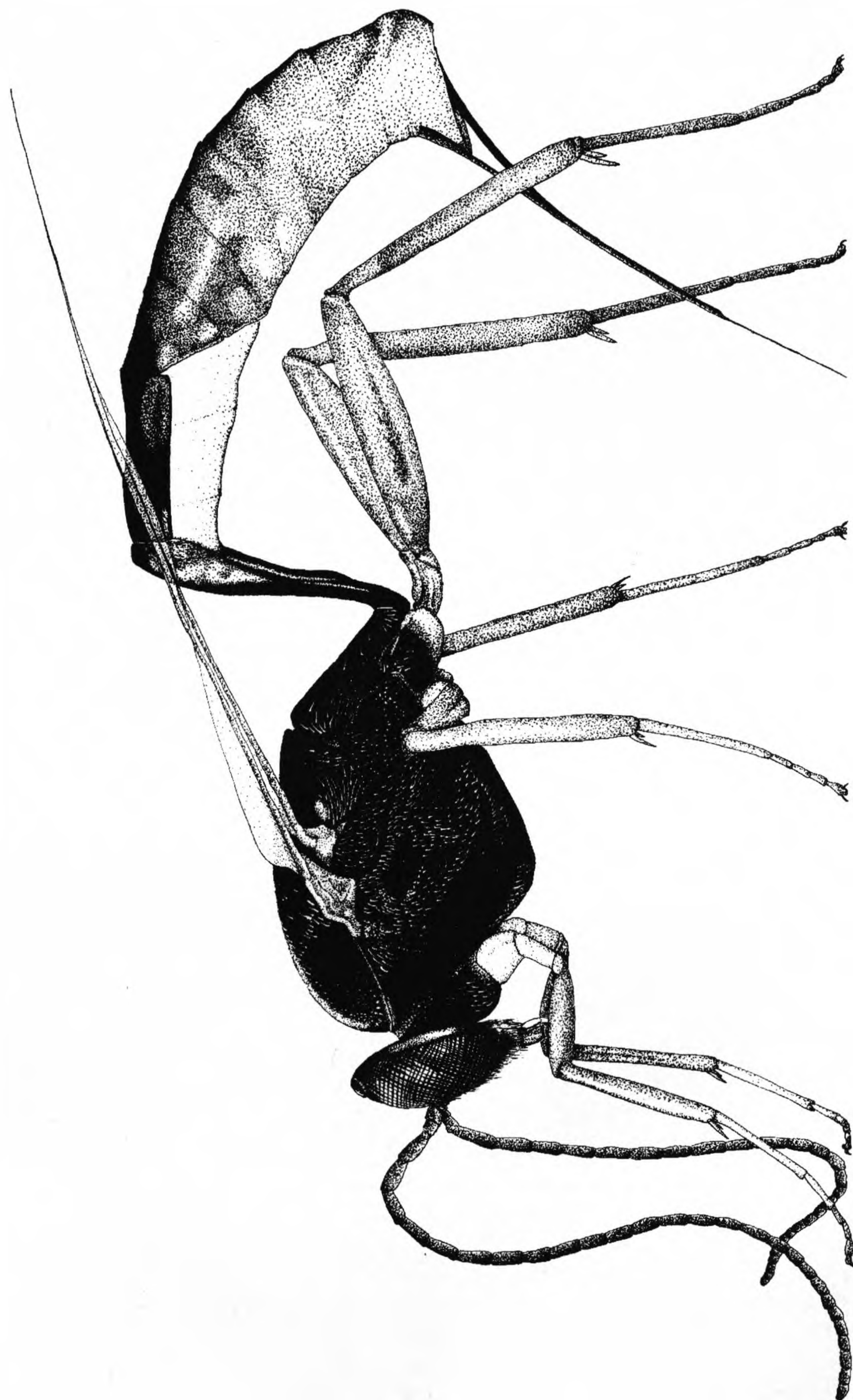

Figure 1.2 *Venturia canescens* adult female in attack posture ×30.

The egg is coated in virus-like particles that probably prevent the host's immune system from encapsulating it (Rotheram, 1967; Beckage, 1997). Once the egg hatches, the parasitoid larva 'drinks' the host's haemolymph to suppress the host's immune system further (Salt, 1968). *V. canescens* is a koinobiont, allowing the host to continue to grow after parasitism. If the host is not large enough for the parasitoid to complete its development, the first instar parasitoid larva becomes dormant, allowing the host to reach full size, before reactivating and growing again (Harvey *et al.*, 1994). If two or more eggs are laid in the same host, the parasitoid larvae will fight until only one remains (Fisher, 1961b). The growing parasitoid passes through 5 instars, and eventually kills the host before pupating inside the body cavity (Corbet and Rotheram, 1965).

1.3.2 Culturing methods

The wasp culturing methods were similar for both the 25°C and the 28°C cultures. The wasps in each culture originated from the same source and were kept under the same conditions as the respective *P. interpunctella* culture. New cultures were set up in clear plastic boxes containing a thin layer (approximately 5mm) of moth food medium in the base. About 100 5th instar *P. interpunctella* and 10 adult *V. canescens* were added to the boxes, and the wasps were left to parasitise the caterpillars until they died. When the new wasps emerged in a box (about 21 days after parasitism), a ball of cotton wool soaked in honey and water solution was added, and the wasps were kept until required.

1.3.3 Handling

The wasps could be handled quite easily using soft forceps, if they were first cooled in a refrigerator (or briefly in a freezer) to make them less active. For parasitising individual hosts, a wasp was placed in a small glass vial placed open end down on a clean surface, then a *P. interpunctella* caterpillar was introduced to the vial and the wasp was watched until it probed the caterpillar with its ovipositor. If the wasp subsequently 'cocked' its ovipositor then it had successfully parasitised the caterpillar (Rogers, 1972).

2. Differential cannibalism

2.1 Introduction

Cannibalism is a common phenomenon in many animal species (Fox, 1975; Polis, 1981), often accounting for very high levels of mortality in a population. The effects of cannibalism on single-species systems have been explored extensively in models of population dynamics (Polis, 1981). However, little attention has been paid to the role of cannibalism in the interactions of two or more species, such as those between a pathogen or parasite and its cannibalistic host.

Cannibalism can be advantageous to the cannibal, since such a highly nutritious diet often leads to increased survival and reproductive potential (Joyner and Gould, 1985). However, cannibalism can also be costly, leading to the risk of injury from the victims' defence, and reducing inclusive fitness if closely related individuals are cannibalised (Pfennig *et al.*, 1993). When diseases or parasites can be acquired through eating infected conspecifics, this can add a strong extra risk to cannibalism (Polis, 1981; Elgar and Crespi, 1992). Transmission of pathogens and parasites by cannibalism has been shown in several species (Schaub, 1988; Matuschka and Bannert, 1989; Schaub *et al.*, 1989; Boots, 1998) and may significantly increase the risk of infection and death (Pfennig *et al.*, 1991). However, Hart (1990) suggests that where diseases or parasites are not transmitted by ingestion of infected tissue, cannibalism may *prevent* the spread of infection by removing infectious individuals from the population. Parasitoids can be useful study animals to test this last theory, since their insect hosts can show high levels of cannibalism, and the parasitoid larvae are killed as the host is eaten, so there is no chance of cross-infection.

In a study of the moth, *Galleria melonella*, and its tachinid parasitoid, *Pseudogonia rufifrons*, Dindo (Dindo and Cesari, 1985; Dindo, 1987; Dindo, 1988) found that parasitism affected the host's cannibalism behaviour. She found that parasitised hosts cannibalised pupae significantly more than unparasitised hosts (Dindo and Cesari, 1985). Cannibalism rates of unparasitised hosts were not affected by crowding or starvation, however both of these factors increased cannibalism by parasitised hosts (Dindo and Cesari, 1985; Dindo, 1987). The rate of cannibalism by parasitised hosts was also affected by the time since

parasitism, with more recently parasitised hosts having lower cannibalism rates (Dindo, 1988).

Dindo's appears to be the only study to date of the effects of parasitism on host cannibalism, but it only considers the cannibal and not the victim. Parasitism may not only affect a host's cannibalistic behaviour, but also its vulnerability to cannibalism. Both of these factors could have significant effects on the population dynamics both of the host and of the parasitoid. I explore the theoretical aspects of this interaction in Chapter 3, but here I describe two experiments designed to test whether parasitised and unparasitised hosts suffered different rates of cannibalism. The first experiment looked at encounters between 2 individuals, while the second experiment used groups of larvae.

2.2 Individual encounters

2.2.1 Methods

The hosts were *Plodia interpunctella* (Hübner) larvae, and the parasitoid was *Venturia canescens* (Gravenhorst). Both were taken from the 25°C cultures (see section 1.2.2). The experiment was conducted at 25°C in a controlled temperature room with a 16:8 hour light:dark cycle, using 1.5ml tapered centrifuge tubes with small air holes punched in the lids.

For the experiment, early fourth instar host larvae were taken from culture and individually parasitised by placing each one under a glass vial with a single *V. canescens*, and waiting until the wasp laid an egg in it. *V. canescens* uses a characteristic 'cocking' motion of its ovipositor after laying an egg (Rogers, 1972), so it was possible to guarantee that each larva had been parasitised. The parasitised larvae were then placed into individual tubes half filled with food. Equal numbers of unparasitised larvae were also set up in individual tubes after being handled in the same way as the parasitised larvae. The larvae were left for four days to develop to early fifth instar stage, by which time the parasitoid larvae should have hatched and begun feeding on the host (Salt, 1968). They were then randomly assigned to new, empty tubes as one of three treatments: 1 — a parasitised and an unparasitised larva together, to test for cannibalism; 2 — a parasitised larva alone, to determine the rate of parasitoid encapsulation; 3 — an unparasitised larva alone, to monitor the mortality rate of larvae during the experiment. The larvae were left for 48 hours for cannibalism to take place in treatment 1, before the number of larvae

remaining alive in each tube was counted and food was added. The larvae were reared through and the number of adult wasps and moths emerging in each tube was counted.

		Treatment		
		1	2	3
Initial numbers of larvae	*Parasitised*	133	133	—
	Unparasitised	133	—	133
Nos. emerging after cannibalism	*Parasitoids*	19	—	—
	Moths	40	—	—
Nos. emerging after no cannibalism	*Parasitoids*	48	108	—
	Moths	64	7	117
Deaths due to cannibalism	*Parasitoids*	40	—	—
	Moths	19	—	—
Non-cannibalism deaths		36	18	16

Table 2.1 Initial numbers of hosts, parasitoid and moth emergence with and without cannibalism, and cannibalism and non-cannibalism mortality for the three experimental treatments (1 = parasitised and unparasitised host together; 2 = parasitised alone; 3 = unparasitised alone).

2.2.2 Results

Cannibalism was assumed to have occurred in treatment 1 (parasitised with unparasitised larvae) if only one larva remained after 48 hours. The results are summarised in Table 2.1. One hundred and twenty-five of the 133 replicates of treatment 1 were successfully reared through to produce adult moths and/or wasps, and cannibalism occurred in 59 of these. In treatment 2 (parasitised larva on its own), 7 out of 115 hosts developed into moths, and can be assumed to have encapsulated the developing parasitoid, giving an encapsulation rate of 0.061. The null hypothesis for the experiment was that parasitism would have no effect on cannibalism, so that equal numbers of parasitised and unparasitised larvae

would survive. After adjusting for the encapsulation rate of parasitoids, this gives expected values of 31.3 moths and 27.7 parasitoids for treatment 1. These expected values are significantly different from the observed numbers emerging (X^2 = 5.15, d.f. = 1, $p<0.03$). Mortality rates of parasitised (treatment 2) and unparasitised (treatment 3) larvae were very similar (X^2 = 0.07, d.f. = 1, $p>0.79$) hence there is no evidence to support the idea that differences in emergence in treatment 1 were due to differential mortality.

2.3 *Density experiment*

2.3.1 Methods

This experiment also used *P. interpunctella* and *V. canescens* taken from the 25°C cultures. Early 4th instar *P. interpunctella* taken from stock culture were parasitised in the same way as in section 2.2.1, but were then immediately placed in 30ml Universal tubes containing 5g food with unparasitised larvae according to the appropriate treatment. The experiment was a full factorial design with 2 factors: density — 8, 16 or 32 larvae in a tube; and relative density — 0%, 25%, 50%, 75%, and 100% parasitised larvae. Thus, for example, a tube with a density of 32 and a relative density of 25% contained 8 parasitised and 24 unparasitised larvae. The tubes were then left for about 30 days, until all of the adult moths and wasps had emerged in them and could be counted. The experiment was repeated as 7 complete blocks of treatments, with blocks being set up about a week apart.

2.3.2 Results

In the following analysis, proportion data (e.g. mortality rates) were arcsine transformed (Sokal and Rohlf, 1995). There were significant block effects on the mortality of parasitised larvae (ANOVA, F = 3.50, d.f. = [6, 77], p = 0.004), however block *did not* affect the mortality of unparasitised larvae (ANOVA, F = 2.05, d.f. = [6, 74], p = 0.07). This overall difference in the response of parasitised and unparasitised larvae between blocks meant that I was unable to analyse the data further in any meaningful way. Tests of treatment effects on mortality were all non-significant once the effects of block had been removed.

2.4 Discussion

The two experiments in this chapter gave rather conflicting results. The first experiment, looking at individual encounters, showed that cannibalism could lead to increased mortality of parasitised hosts. However, in the second experiment, using groups of larvae, there were no effects of parasitism on mortality, or they were obscured by block effects. The strange effects of block on mortality in the second experiment may have been caused by *Mattesia dispora* infection of the *P. interpunctella* larvae used for the later blocks of the experiment (see section 1.2.6). Although the infection of the cultures was not identified until much later, it is clear with hindsight that they were already showing signs of the infection — as reduced fecundity and increased mortality — while I was completing the second experiment. I repeated this experiment on a larger scale soon afterwards, but by this time the *M. dispora* infection was more widespread in the moth cultures, so that nothing survived in any of the treatments and I had to abandon the experiment altogether.

The characteristics of cannibalism in my experiments differ from those in Dindo's study of *Galleria melonella* and *Pseudogonia rufifrons* (Dindo and Cesari, 1985; Dindo, 1987; Dindo, 1988). Here, the larvae were cannibalising each other, rather than pupae, thus there was a significant risk that the cannibal may have become the victim. Moreover, there was no difference in the basal rates of cannibalism between parasitised and unparasitised hosts, however, cannibalism rates of both types of hosts increased as density increased. This was different from the situation in the *G. melonella* and *P. rufifrons* system, where parasitised and unparasitised hosts showed different responses to crowding and starvation (Dindo and Cesari, 1985; Dindo, 1987).

In order to encourage cannibalism to take place, little or no food was made available to the *P. interpunctella* larvae during these experiments. It has been suggested that cannibalism is often a laboratory artefact, caused by extreme conditions of starvation or density (Fox, 1975), however some studies have shown that cannibalism would occur even in the presence of abundant food and low density (Dial and Adler, 1990; Van den Bosch and Santer, 1993). My own observations suggested that this was also the case for *P. interpunctella*.

The experimental results did not show how the differential cannibalism found in the first experiment came about. Potential mechanisms would probably involve either a change in

the behaviour of parasitised larvae, making them more vulnerable to cannibalism, or detection and preferential attack of parasitised larvae by cannibals. An immature parasitoid can have profound effects on the behaviour and development of its host (Godfray, 1994 ch. 6), and this could affect the vulnerability of the host to cannibalism. *V. canescens* is known to coat its eggs in virus-like particles which weaken the host's immune system and prevent encapsulation (Rotheram, 1967). Once the parasitoid larva hatches it rapidly feeds on the host's haemolymph, which depresses its immune system even further, and forces the host to use up fat reserves to compensate (Salt, 1968). These effects will probably combine to weaken the host larva, making it less able to resist attack by cannibals, and less likely to attack other larvae. The parasitoid may also slow down the host's development by depleting resources, making it smaller than an unparasitised larva of the same age (Harvey *et al.*, 1994). Smaller individuals are usually more susceptible to cannibalism, but this situation can be reversed if the larger individual still has a soft cuticle after moulting, and so is more vulnerable to attack (Dial and Adler, 1990). Finally, cannibals may preferentially attack parasitised individuals. Such a mechanism would involve detection of the parasitoid larva within a parasitised host by the cannibal, possibly using chemical or behavioural cues.

Whichever mechanism is responsible for the differential cannibalism found in the first experiment, the end result is that the number of parasitised individuals in a population of hosts will be reduced compared to a population in which no differential cannibalism occurs. This, in turn, will lead to fewer parasitoids present in the next generation to parasitise hosts. Thus, the results of the first experiment support Hart's theory (1990) that cannibalism could, in some cases, control the spread of parasitism. The costs of differential cannibalism are more difficult to determine, however, since some of the normal costs of cannibalism do not apply in this case. For instance, once a *P. interpunctella* larva has been parasitised it will almost certainly die — fewer than 7% of the parasitised larvae in the first experiment were able to encapsulate the parasitoid and develop normally — so even if the cannibal is closely related to its victim it does not suffer an appreciable loss in inclusive fitness through cannibalism. Also, the cannibal is probably less likely to be injured attacking a weakened, parasitised victim, so the main potential cost for the cannibal will not be injury, but rather the risk of becoming infected with other forms of disease or parasite which can be transmitted during cannibalism.

3. Modelling cannibalism

3.1 Introduction

Cannibalism is a widespread phenomenon and is likely to be an important factor in regulating population size (Fox, 1975; Polis, 1981; Elgar and Crespi, 1992). In this chapter I will review existing models that examine the effects of cannibalism on the population dynamics of one- and two-species systems, before going on to develop new models that include host cannibalism in a host-parasitoid interaction.

3.2 Approaches to modelling cannibalism

3.2.1 The characteristics of cannibalism

In many ways, cannibalism is simply a special case of a predator-prey relationship (Dong and Polis, 1992), since any cannibalistic interaction involves a cannibal (predator) and a victim (prey). However, this simple picture is clouded by the highly dynamic nature of cannibalism, where the distinction between potential cannibal and potential victim can sometimes be hard to define. Although both cannibal and victim are the same species, they need not be the same developmental stage — e.g. egg cannibalism by larvae and adults (Stevens, 1989; Pajunen and Pajunen, 1991) or cannibalism between different larval instars (Wissinger, 1988). A species may show a cannibalistic polyphenism (Polis, 1981; Elgar and Crespi, 1992) — a distinction between cannibalistic and non-cannibalistic morphs — but while there may be an underlying genetic basis (Stevens, 1989; Richter, 1990; Maret and Collins, 1994), the expression of this polyphenism is usually also affected by environmental and population density factors (Polis, 1981; Collins and Cheek, 1983; Elgar and Crespi, 1992; Maret and Collins, 1994).

Cannibalism has sometimes been described as a 'life-boat strategy' (Polis, 1981; Cushing, 1992) — a last resort used by an individual when it is unable to find any other sources of food. Hence, the number of potential cannibals in a population may depend upon the availability of alternative food sources and other factors, such as population density, that might affect food supply (Elgar and Crespi, 1992). The distinction between cannibal and potential victim can also become blurred when one considers cannibalistic encounters on

an individual basis. The victor in a cannibalistic interaction between a pair of individuals is usually the larger of the pair (Semlitsch and West, 1988; Smith, 1990; Amundsen *et al.*, 1995), although this situation can sometimes be reversed when larger individuals have just moulted and are more vulnerable to attack (Dial and Adler, 1990). Some of the conspecifics that an individual meets are likely to be smaller than itself while others will be larger, so an individual will oscillate between being a potential cannibal and a potential victim from moment to moment. The upshot of this is that mortality rates from cannibalism in a population will depend not only on the population density and food availability, but also on the size distribution of individuals and the likelihood of individuals of different sizes encountering each other.

The inclusion of all of these different aspects of cannibalism in a model of population dynamics would probably make it impossible to analyse in any sensible form, so cannibalism models are usually hedged with many simplifying assumptions. The two main approaches are (1) to model cannibalism as an extra (often density-dependent) mortality factor in an existing population model, and (2) to develop a model based on optimal foraging theory, where cannibalism becomes a problem of appropriate prey selection for the cannibal.

3.2.2 Cannibalism as density-dependent mortality

Perhaps the easiest way to model cannibalism is to include it as an extra mortality factor in an existing population state model. This approach has to make many simplifying assumptions about the nature of cannibalism in a population, but it does lead to tractable solutions that can still give a good idea of the general effects that cannibalism might have on a population. Bernstein (1986) developed a discrete time, host-parasitoid model that allowed differences between the host and parasitoid in their susceptibility to density-dependent mortality. He found that both general increases in density-dependent mortality, and increases in the mortality of parasitoids, reduced the region where stable coexistence of host and parasitoid was possible.

The effects of cannibalism on the stability of one species systems have been explored in several studies. These models show that the stage at which cannibalism occurs can affect whether the system is stable or oscillates. In *Tribolium*, adult cannibalism of larvae (Hastings and Costantino, 1987), and adult cannibalism of eggs and pupae (Desharnais and Liu, 1987) are stabilising, while larva-larva and larva-egg cannibalism lead to oscillations

(Desharnais and Liu, 1987; Hastings and Costantino, 1987; Hastings and Costantino, 1991). Landahl and Hansen (1975) found that larva-larva cannibalism could lead to stability or oscillations depending upon parameter values, while Diekmann *et al.* (1986) found egg cannibalism always lead to oscillations.

In two-species systems, the effect of cannibalism is nearly always stabilising. Kohlmeier and Ebenhöh (1995) have found that predator cannibalism is generally stabilising in a predator-prey system, while Crowley and Hopper (1994) show that very low prey levels can lead to oscillations. Bernstein (1986) finds that density-dependent mortality is stabilising in a host-parasitoid model, while May *et al.* (1981) show that, as well as being stabilising, density-dependence that acts after parasitism can *increase* equilibrium host and parasitoid levels.

3.2.3 Cannibalism as a foraging problem

Dong and Polis (1992) suggest that foraging theory provides a good starting point for investigating the possible effects of cannibalism on population dynamics. They argue that an individual animal's decision on whether or not to cannibalise is a problem of diet selection and will affect, and be affected by, the availability of alternative food sources. Few models have adopted this approach, though, and Dong and Polis (1992) provide a framework for future models using an individual-based method. One set of individual-based models of young-of-the-year largemouth and smallmouth bass (DeAngelis *et al.*, 1979; DeAngelis *et al.*, 1991) show that these individual-based approaches can model very accurately the effects of cannibalism within a single season.

3.3 Model A: a Lotka-Volterra host-parasitoid population model with cannibalism

This model was initially developed by Prof. M. Begon to explore the potential population dynamic effects of the differential cannibalism found by the experiment described in section 2.2 and appeared in Reed *et al.* (1996). I shall refer to it as 'model A' to distinguish it from other variations introduced later in this chapter. It is based on the continuous time, predator-prey model originally developed by Lotka and Volterra, and adapted for a host-parasitoid system:

$$\frac{dH}{dt} = rH - aHP;$$

Equation 3.1

$$\frac{dP}{dt} = faHP - mP.$$

Equation 3.2

In the host equation (Equation 3.1) the hosts (abundance *H*) have an intrinsic rate of increase (*r*) and are attacked by parasitoids (abundance *P*) with an attack rate (*a*) that is proportional to the numbers of hosts and parasitoids. The parasitoid equation (Equation 3.2) takes the parasitised hosts from Equation 3.1 and converts them into parasitoids with a certain conversion efficiency (*f*), while the number of parasitoids is kept in check by a density-independent mortality (*m*).

The Lotka-Volterra model assumes that both host and parasitoid have continuous, overlapping generations, and exponential distributions of development times. In the absence of the parasitoid, host numbers will increase exponentially, while in the absence of the host, parasitoid numbers will decrease exponentially. This set of equations generates coupled, neutrally stable population cycles for both the host and parasitoid.

The new model adds extra terms to the basic Lotka-Volterra formulation to give new host (Equation 3.3) and parasitoid (Equation 3.4) equations:

$$\frac{dH}{dt} = rH - qH^2 - aHP - c_H H^3;$$

Equation 3.3

$$\frac{dP}{dt} = faHP - mP - c_P H^2 P.$$

Equation 3.4

The host numbers in Equation 3.3 are now controlled by a density-dependent crowding factor (*q*) so that in the absence of the parasitoid, host numbers increase asymptotically to

a carrying capacity. The new model also adds terms to both the host and parasitoid (Equation 3.4) equations that account for the mortality caused by host cannibalism. For the host, this cannibalism term consists of a cannibalism coefficient (c_H) multiplied by the host density, H, to give a density-dependent cannibalism vulnerability, $c_H H$. This is then combined with an encounter rate between hosts, H^2, to give the $-c_H H^3$ term. The parasitoid cannibalism term is broadly similar except that a different cannibalism coefficient (c_P) is used — to allow differences in the vulnerability of parasitised and unparasitised hosts to cannibalism — and the encounter rate is between parasitised and unparasitised hosts, HP, giving the $-c_P H^2 P$ term.

Note that this model assumes that *only* unparasitised hosts cannibalise both other unparasitised and parasitised hosts. As in the original Lotka-Volterra equation, it considers larvae and adults of each species within one equation, which may oversimplify the situation, since density-dependent factors, like host crowding and cannibalism, will affect only the larvae. Despite these limitations, the model does give a useful starting point for exploring the possible effects of cannibalism on two-species population dynamics.

The behaviour of the model is explored most easily by looking at the zero isoclines for the host and parasitoid. The zero isoclines represent values of host and parasitoid abundance where there is no change in density with time. When the two sets of isoclines are overlaid, they can be used to explore the dynamics of the two-species system. The isoclines can be found by solving Equation 3.3 and Equation 3.4 for dH/dt and $dP/dt = 0$ and rearranging them to give the following:

host zero isocline $$P = \frac{c_H}{a} H^2 - \frac{q}{a} H + \frac{r}{a};$$

Equation 3.5

parasitoid zero isocline $$H = \frac{fa \pm i}{2c_P};$$

Equation 3.6

where $$i = \sqrt{f^2 a^2 - 4c_P m}\ .$$

Equation 3.7

The host zero isocline is a straight line when $c_H = 0$, and a simple curve in the region where P and H are both positive (Figure 3.1a), when $c_H > 0$. When P is zero the host stabilises at a carrying capacity:

$$H = \frac{-q + \sqrt{q^2 + 4c_H r}}{2c_H}.$$

Equation 3.8

There are two parasitoid isoclines (Figure 3.1b) which run parallel to the parasitoid axis (i.e. H is constant) and which divide alternating regions where P decreases, increases and then decreases again with progressively greater values of H. The two isoclines combine when $i = 0$, while as c_P tends to zero, the right-hand isocline moves towards infinite H, leaving the single parasitoid isocline of a normal Lotka-Volterra model. To rephrase in more biological terms, at low values of H, parasitoid densities decrease because there are not enough hosts to support a viable population. Past the first parasitoid isocline, there are now enough hosts to support parasitoids, so P increases. However, past the second isocline, the effects of host cannibalism are great enough to depress parasitoid density again. When $i = 0$, the mortality from the combination of cannibalism, c_P, and adult death rate, m, is great enough to counteract parasitoid recruitment (governed by attack rate, a, and conversion efficiency, f). When this happens, the two parasitoid isoclines combine and there is no region of parasitoid increase.

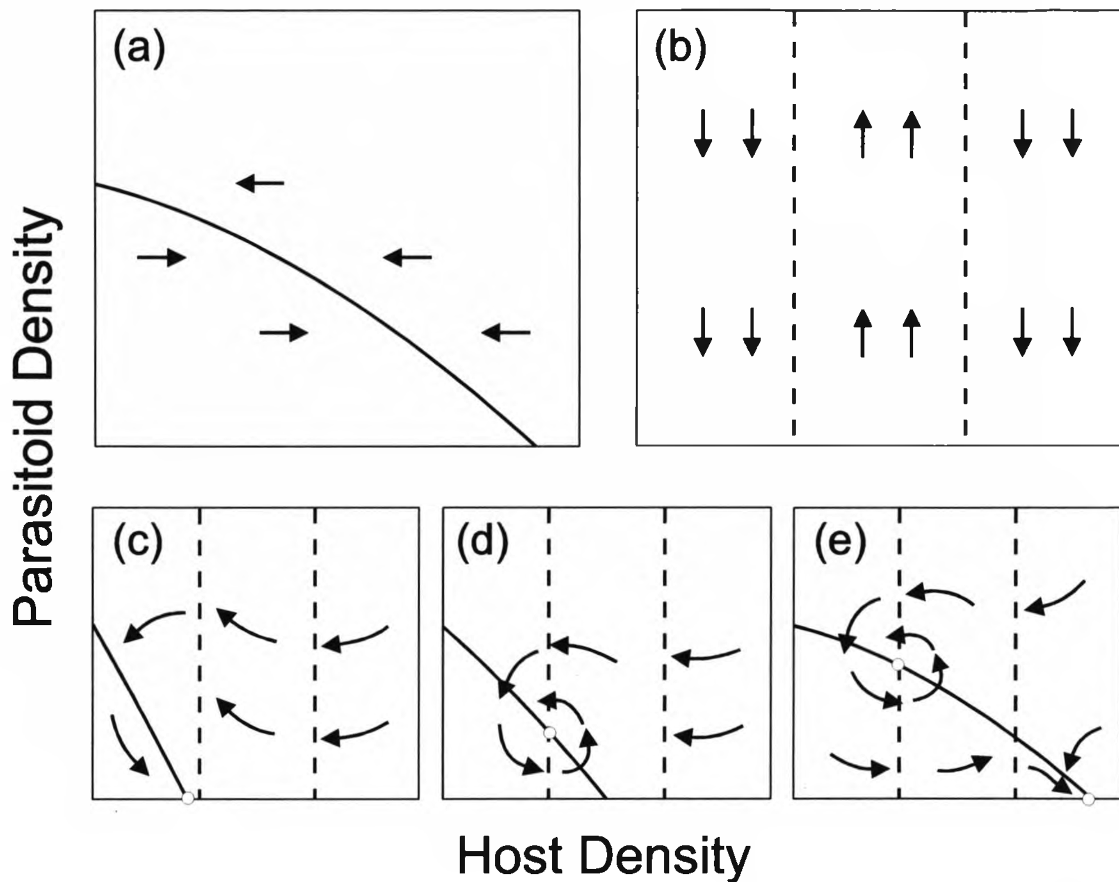

Figure 3.1 The zero isoclines for model A described in section 3.3, with arrows indicating directions of flow. **(a)** The host zero isocline (solid line); **(b)** the parasitoid zero isoclines (dashed lines). **(c-e)** Show the different ways in which the host and parasitoid isoclines can be juxtaposed: **(c)** the system settles (**O**) at the host's carrying capacity with no parasitoids; **(d)** the system approaches an equilibrium point (via damped cycles) at the point where the host and lower parasitoid zero isocline meet; **(e)** the outcomes of **(c)** and **(d)** are alternative stable states dependent upon starting conditions.

The host and parasitoid isoclines can be juxtaposed in three different ways — dependent mainly upon the levels of host cannibalism and parasitoid searching efficiency — each leading to different qualitative outcomes (Figure 3.1c-e). If both parasitoid isoclines lie beyond the point where the host isocline meets the host axis (i.e. the host-alone carrying capacity) then the only stable outcome is the parasitoid absent and the host at its carrying capacity (Figure 3.1c). If the first of the parasitoid isoclines crosses the host isocline (Figure 3.1d) then the system reaches a stable equilibrium, via damped, coupled oscillations, at the point where they cross. In this case, the parasitoid searching efficiency is high enough for parasitoids to persist below the host's carrying capacity, while host cannibalism is not enough to depress parasitoid numbers to extinction. The third

possibility is that both parasitoid isoclines cross the host isocline (Figure 3.1e), in which case there are two alternative stable outcomes that are dependent upon the starting densities of hosts and parasitoids. If the starting density of parasitoids is high enough, then the system settles at stable coexistence of the host and parasitoid, as before, but if the starting density of parasitoids is too low, then the levels of host cannibalism are enough to depress the parasitoid to extinction, leaving the host stable at its carrying capacity.

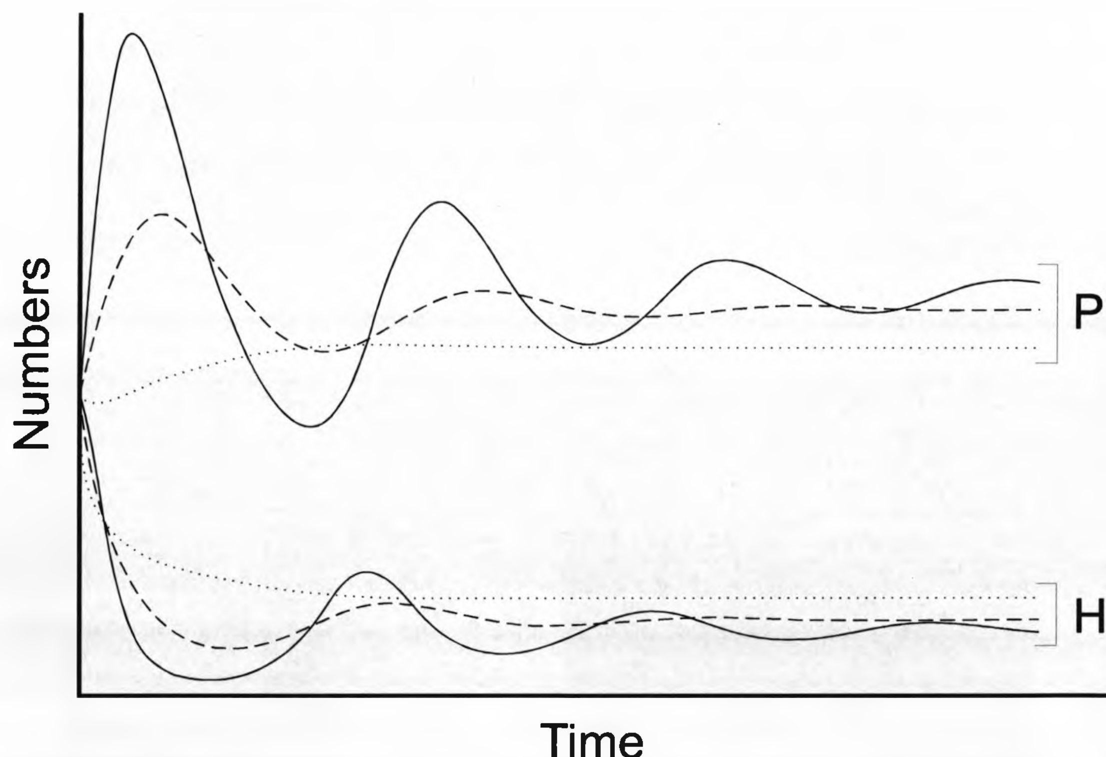

Figure 3.2 Time series from model A, showing the stabilising effect of cannibalism. As levels of cannibalism increase from 0 (solid line) to $c_H = c_P = 0.1$ (dashed line) to $c_H = c_P = 0.2$ (dotted line), host (H) and parasitoid (P) numbers reach equilibrium sooner and with fewer oscillations. Values of other parameters in the model were: a = 0.4; f = 1; m = 0.175; q = 0.3; r = 1.2. The starting values of H and P were both 2.

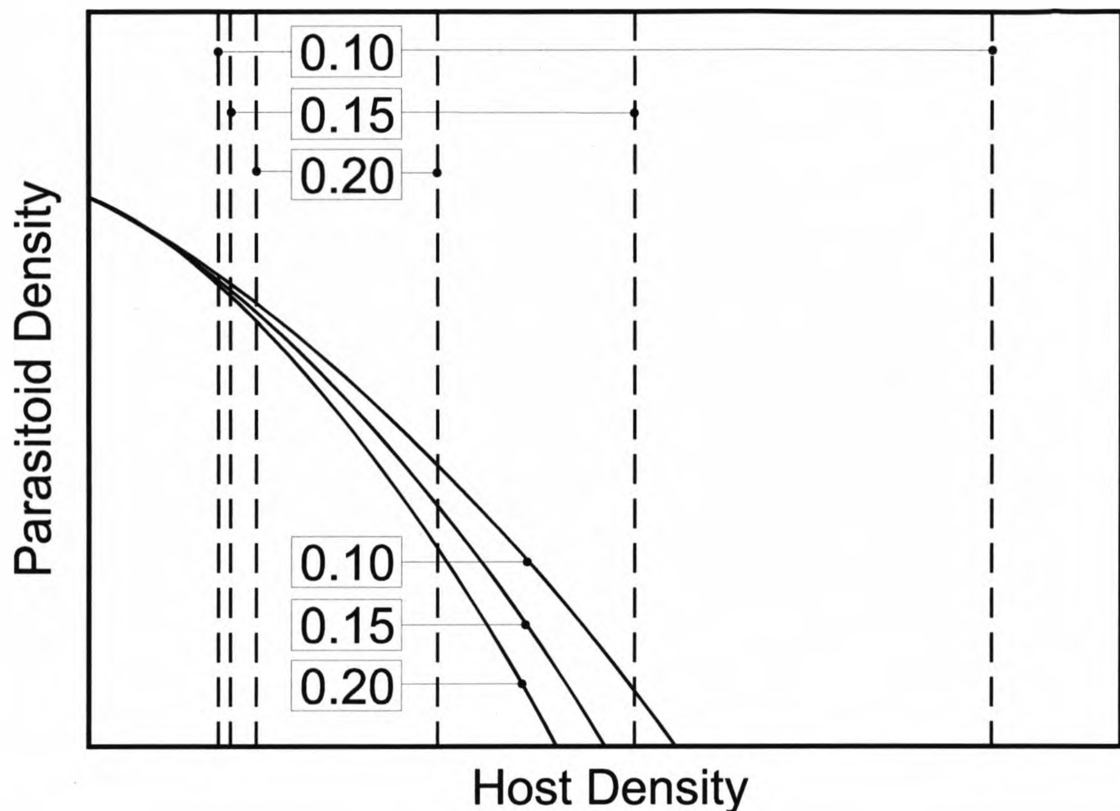

Figure 3.3 Phase plane diagram demonstrating the effects of different levels of cannibalism on the host (solid lines) and parasitoid (dashed lines) zero isoclines in model A. Numbers are values of c_H (for the host isoclines) and c_P (for the parasitoid isoclines). Small changes in the vulnerability of the host to cannibalism (c_H) affect the position of the host isocline relatively little. However, small changes to the parasitoid's cannibalism vulnerability (c_P) result in large shifts in the positions of the parasitoid zero isoclines, and especially the position of the right-hand isocline. Values of other parameters were the same as for Figure 3.2.

The general effect of cannibalism is to stabilise the system. As levels of cannibalism increase, the two species reach an equilibrium more quickly and with fewer oscillations (Figure 3.2). This agrees with other population models which show that cannibalism is stabilising. Note that the level of cannibalism has relatively little effect on the equilibrium densities of host and parasitoid. Despite the overall stabilising influence of cannibalism, it does lead to the possibility of alternative stable states, as shown in Figure 3.1e. This situation can arise either when there are generally high levels of cannibalism (c_H and c_P both large) or when there is differential cannibalism ($c_P > c_H$). The positions of the parasitoid zero isoclines are more sensitive to changes in the level of cannibalism than the host zero isocline (Figure 3.3), thus as c_P increases, the system quickly moves into the region where alternative stable states are possible.

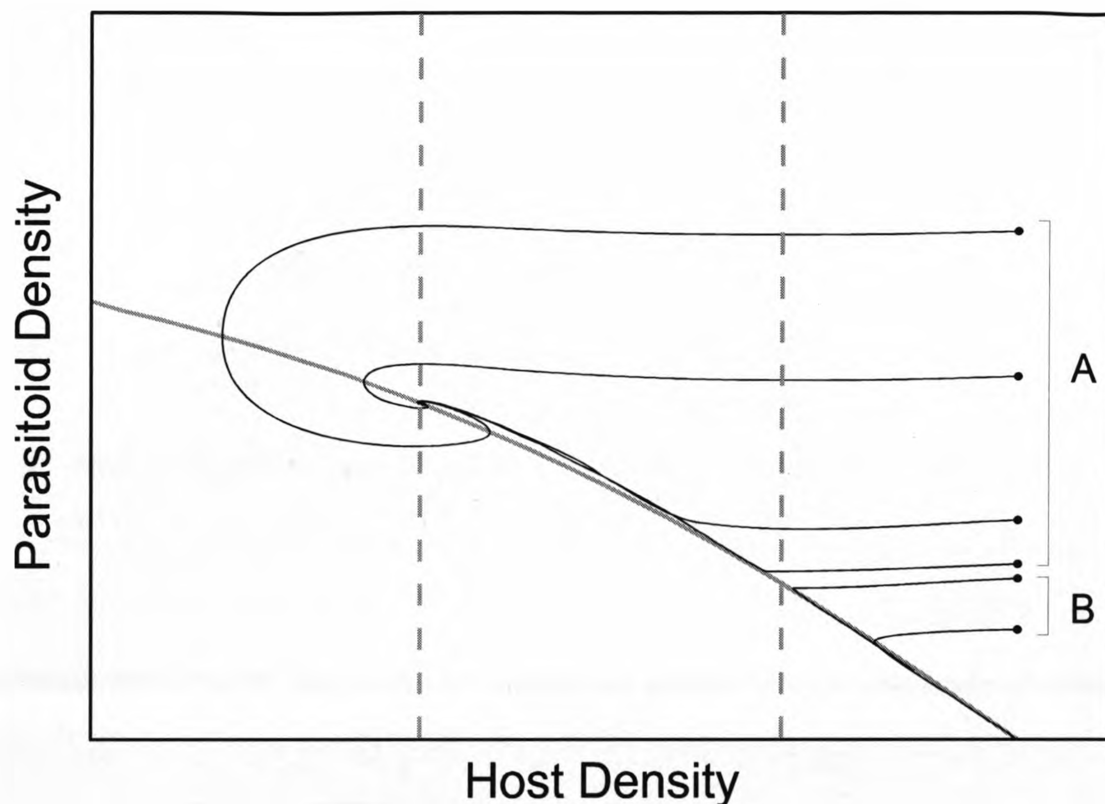

Figure 3.4 Phase plane diagram showing the effects of adding different densities of parasitoids at the host's carrying capacity for model A. Trajectories (●—) from high densities of parasitoids (A) converge on stable coexistence of host and parasitoid, while additions of low densities of parasitoids (B) lead to extinction of the parasitoid. Zero isoclines for the host (solid line) and parasitoid (dashed line) are shown in grey. Parameter values are the same as Figure 3.2 with $c_H = c_P = 0.2$.

The point where the second parasitoid isocline crosses the host isocline is an unstable equilibrium that marks a threshold between the two alternative stable states. Figure 3.4 shows the effect of this threshold on additions of parasitoids when the host is at its carrying capacity. If too few parasitoids are added they never become established and become extinct due to mortality from host cannibalism. Figure 3.5 shows that this threshold is, in fact a line across phase space showing that low density additions of parasitoids at *any* host density will result in the parasitoid becoming extinct. It is not clear what causes this rather surprising result, but it may be an artefact of the way this particular model is constructed.

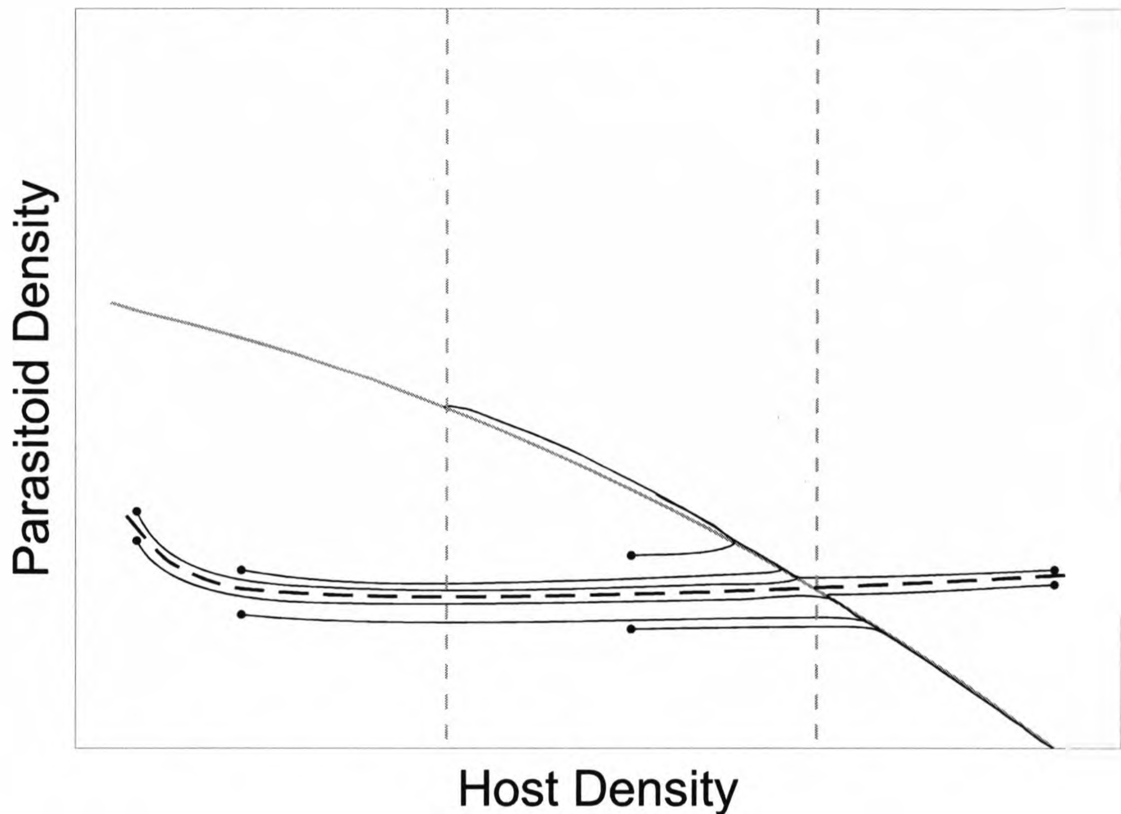

Figure 3.5 Phase plane diagram illustrating the approximate position of the threshold (heavy dashed line) between the two alternative stable states in model A. Trajectories (●—) started above and below the threshold line end at different stable states. Zero isoclines for the host (solid line) and parasitoid (dashed line) are shown in grey. Parameter values are the same as in Figure 3.4.

3.4 Extending the model

3.4.1 Addressing assumptions and considering density-dependence

Section 3.4 builds upon model A, described in section 3.3, by addressing two of its main assumptions: that the density-dependence of cannibalism is only relative to the density of healthy (unparasitised) hosts, and that only healthy hosts are cannibals. As mentioned earlier, there are potential problems with considering density-dependence in such a simple two equation system, since the adults and juveniles of each species are considered within the same equation. Density-dependent factors like cannibalism are likely to act only on the juveniles of each species in a host-parasitoid system, since the adults tend to be short-lived and rarely eat. For these simple models to include density-dependence and still be reasonably realistic, the numbers of parasitised hosts must be roughly equal to the

numbers of adult parasitoids emerging from them. In other words, the conversion efficiency of parasitoids (f in Equation 3.4) must be approximately one. This is probably a reasonable assumption for solitary parasitoids like *V. canescens* for which these models were developed. Although limiting the scope of these models to solitary parasitoids also limits their generality, it does allow a reasonably tractable analysis of the possible effects of host cannibalism on a host-parasitoid system. I will discuss alternative ways to model cannibalism more explicitly and generally later in this chapter, but these methods significantly increase the complexity of models and of their analysis.

3.4.2 Basic form

The basic equations for the models that follow can be written in the general form:

$$\frac{dH}{dt} = rH - qH^2 - aHP - X_H;$$

Equation 3.9

$$\frac{dP}{dt} = aHP - mP - X_P.$$

Equation 3.10

Where X_H is the mortality of healthy hosts from cannibalism and X_P is the mortality of parasitised hosts from cannibalism. The only differences between the model variations that follow are the values of X_H and X_P. As I am only considering solitary parasitoids in these models, $f \approx 1$ in the parasitoid equation and so is ignored (see above).

3.4.3 Healthy host density-dependence and healthy host cannibalism: model A revisited

In the original model A (Equation 3.3 and Equation 3.4), the values of X_H and X_P are:

$$X_H = c_H H^3;$$

Equation 3.11

$$X_P = c_P H^2 P \,.$$

Equation 3.12

Only healthy hosts cannibalise and rates of cannibalism are only dependent on the density of healthy hosts. This form of the model has already been explored in depth in section 3.3, but I will quickly recap for comparison with what follows. The host zero isocline is a curve in phase space that reaches the host axis at the host's carrying capacity, while the parasitoid has two isoclines that are straight lines running parallel to the parasitoid axis. The juxtaposition of these isoclines determines whether the system settles at a stable coexistence equilibrium or with the host stable at its carrying capacity and the parasitoid extinct. When both of the parasitoid isoclines cross the host isocline then the two possible outcomes are alternative stable states that depend on the starting density of the parasitoid: if the starting parasitoid density is low then the parasitoid becomes extinct, while if it is high, the parasitoid becomes established and the system settles at a stable coexistence equilibrium.

3.4.4 Model B: full density-dependence

For the first variation on the basic model, I make the cannibalism density-dependence relative to the combined density of healthy *and* parasitised hosts ($H + P$). It is still only healthy hosts that cannibalise, however:

$$X_H = c_H (H + P) H^2 \,;$$

Equation 3.13

$$X_P = c_P (H + P) HP \,.$$

Equation 3.14

Solving these equations to find the zero isoclines produces a rather unwieldy set of equations, and it proved easier to explore the characteristics of the model using numerical methods. This was done using 'PhasePlane', a program for the IBM-PC which is able to draw phase plane diagrams and zero isoclines using one of several numerical solution algorithms (all of the diagrams for this chapter were produced with this program, using a Runge-Kutta algorithm).

Figure 3.6a and b show the host and parasitoid zero isoclines (for positive values of H and P) in model B and how they are affected by changes in the cannibalism parameters, c_H and c_P. When $c_H = 0$, the model is the same as model A with no cannibalism, so once again, the host isocline is a straight line running diagonally through the parasitoid and host axes. However, when $c_H > 0$ the host isocline becomes a slight concave curve (this is confirmed by exploring negative values of H and P), and this is in contrast to the convex curve of model A. Thus, making cannibalism rates dependent upon the densities of both unparasitised and parasitised hosts reduces the region below the host isocline where host densities can increase. This is because, as well as parasitism, the parasitoid can now affect host mortality through the crowding effect of parasitised hosts leading to higher rates of cannibalism. As the value of c_H increases, the host isocline reaches the host axis (the host's carrying capacity) at progressively lower values of H, as increased mortality from cannibalism limits the possible maximum host density. However, the point where it crosses the parasitoid axis is unaffected, since in this region, the cannibalism of parasitised hosts by the very few healthy hosts in the population will be insignificant.

There is only one parasitoid zero isocline: when $c_P = 0$ it is a straight line parallel to the parasitoid axis, as in model A, while when $c_P > 0$, the parasitoid isocline becomes a curve that crosses the host axis twice. As c_P increases, the maximum height of the curve, and the point where it re-crosses the host axis are successively reduced until the isocline disappears altogether for positive values of H and P. This is very different to model A, where for $c_P > 0$ there are two vertical isoclines that grow closer together as c_P increases.

In model A, parasitoid mortality is completely independent of parasitoid density, and instead depends only on the density of healthy hosts. At low values of H there are too few hosts to support a viable population of parasitoids, while at high host densities, the level of cannibalism also suppresses the parasitoid. It is only between these two extremes of host density that the parasitoid can increase, regardless of the parasitoid abundance, hence the two vertical parasitoid isoclines. In the current model, however, the density of parasitised hosts *does* have an effect on levels of cannibalism. At a given value of H, as the number of parasitised hosts increases, they encourage greater rates of cannibalism by the unparasitised hosts, until the level of cannibalism is enough to prevent any further increase in parasitoid numbers.

The exact shape of the parasitoid isocline comes from the interaction of the number of parasitised hosts contributing to the density-dependence of cannibalism and the number of healthy hosts that are able to cannibalise. When there are few healthy hosts (low H), there are few cannibals, and the parasitoids are mainly limited by their searching efficiency, hence the steep rising edge of the curve and the small effect of c_P on the point where the isocline springs from the host axis. At higher levels of H, there are more potential cannibals in the population, but it still takes a high density of parasitised hosts before the rate of cannibalism is enough to limit parasitoid increase. At even higher values of H, the number of healthy hosts contributes a significant portion of the total density ($H + P$) that in turn affects cannibalism levels. Thus it takes fewer parasitised hosts before the rate of cannibalism is enough to stop P increasing. Eventually, the density of healthy hosts is high enough for the level of cannibalism to limit parasitoid increase even when there are no parasitised hosts, and at this point the parasitoid isocline meets the host axis again. The maximum height of the parasitoid zero isocline and the point where it re-crosses the host axis depend upon the value of the cannibalism vulnerability, c_P, since this affects the density ($H + P$) at which cannibalism mortality is enough to limit parasitoid increase.

Figure 3.6c shows how the host and parasitoid zero isoclines can be combined. The shape of the parasitoid zero isocline is such that it only ever crosses the host isocline once or not at all, therefore, for any set of values of c_H and c_P, there can only ever be one stable outcome (either host and parasitoid coexisting, or parasitoid extinct and the host at it's carrying capacity). This is different from model A, where for some values of c_H and c_P, it was possible to have alternative stable states dependent upon starting conditions. As general levels of cannibalism increase ($c_H = c_P$), the equilibrium densities of host and parasitoid (numbered circles in Figure 3.6c) are greatly affected. This again is different to model A, where changes in c_H and c_P had relatively little effect on equilibrium densities.

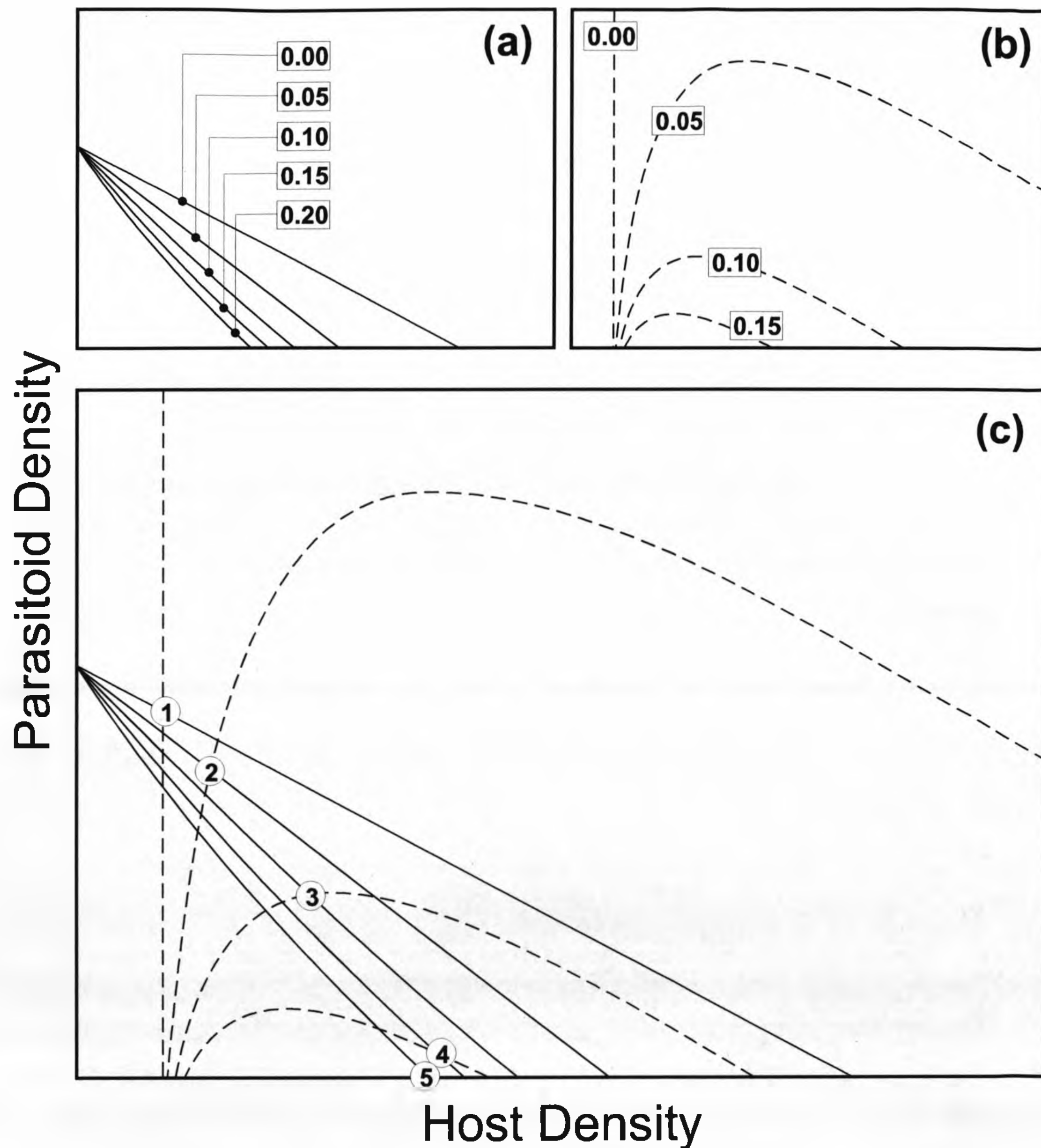

Figure 3.6 Phase plane diagrams for model B. **(a)** Host zero isoclines for different values of c_H (values of c_H shown); **(b)** Parasitoid zero isoclines for different values of c_P (values of c_P shown); **(c)** The host and parasitoid isoclines from **(a)** and **(b)** combined. The numbered circles represent stable equilibria for different values of c_H and c_P: ① $c_H = c_P = 0$; ② $c_H = c_P = 0.05$; ③ $c_H = c_P = 0.10$; ④ $c_H = c_P = 0.15$; ⑤ $c_H = c_P = 0.20$ (parasitoid zero isocline does not rise above the host axis when $c_P = 0.2$). Values of other parameters in the model were: $a = 0.4$; $f = 1$; $m = 0.175$; $q = 0.3$; $r = 1.2$.

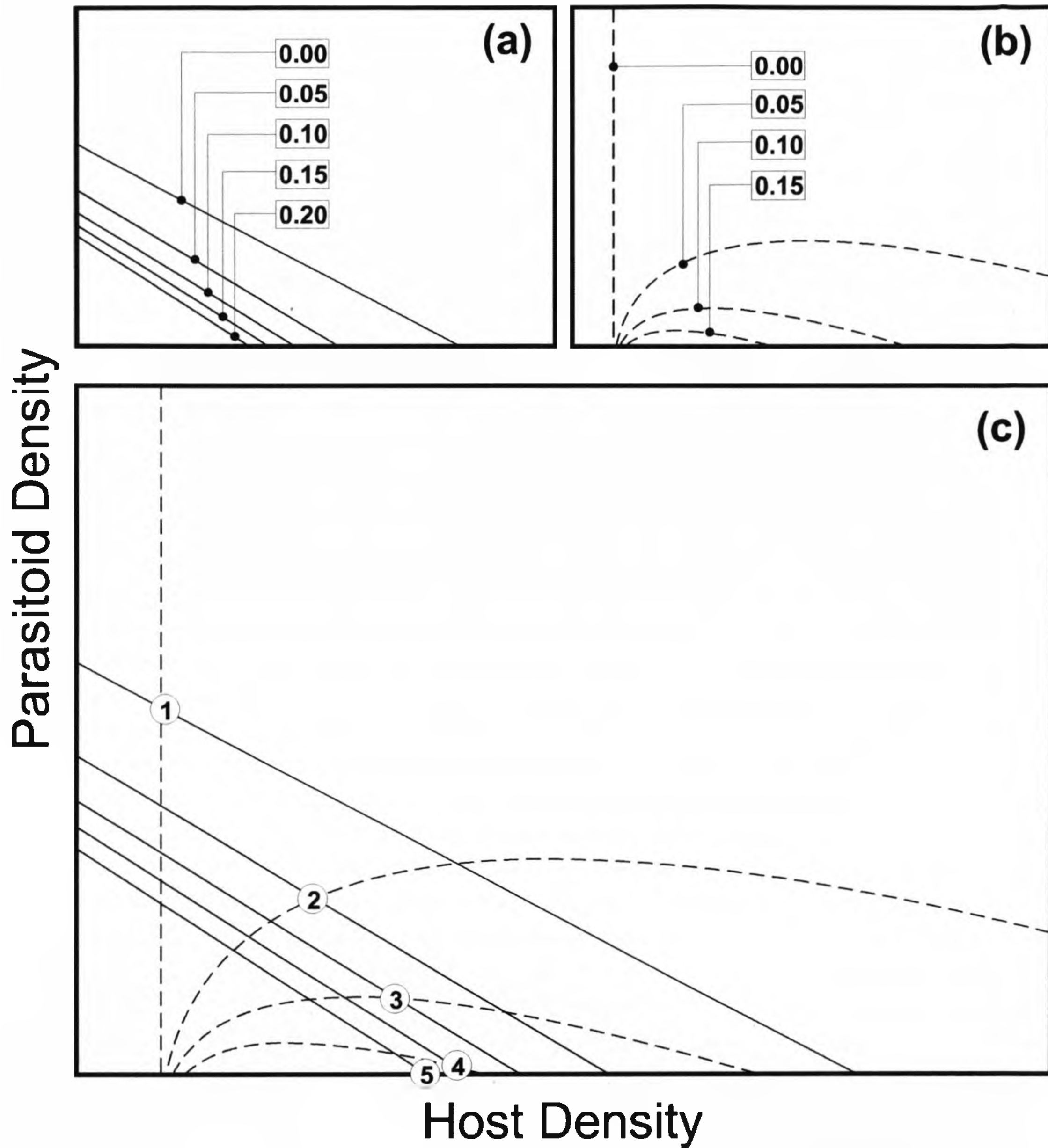

Figure 3.7 Phase plane diagrams for model C shown to the same scale as Figure 3.6 for comparison with model B. **(a)** Host zero isoclines for different values of c_H (values of c_H shown); **(b)** Parasitoid zero isoclines for different values of c_P (values of c_P shown); **(c)** The host and parasitoid isoclines from **(a)** and **(b)** combined. The numbered circles represent stable equilibria for different values of c_H and c_P: ① $c_H = c_P = 0$; ② $c_H = c_P = 0.05$; ③ $c_H = c_P = 0.10$; ④ $c_H = c_P = 0.15$; ⑤ $c_H = c_P = 0.20$ (parasitoid zero isocline does not rise above the host axis when $c_P = 0.2$). Values of other parameters in the model were as in Figure 3.6.

3.4.5 Model C: full density-dependence and full cannibalism

This variation adds to model B, by allowing both healthy *and* parasitised hosts to be cannibals:

$$X_H = c_H(H+P)H^2 + c_H(H+P)HP$$

Equation 3.15

$$X_P = c_P(H+P)P^2 + c_P(H+P)HP$$

Equation 3.16

Cannibalism mortality for each species now consists of two terms, one for cannibalism by healthy hosts and one for cannibalism by parasitised hosts. So, for the healthy hosts (Equation 3.15), both terms are multiplied by the density-dependent, healthy host cannibalism vulnerability $c_H(H + P)$, but one term has an encounter rate for healthy hosts, H^2, and in the other term the encounters are between healthy and parasitised hosts, *HP*. The parasitoid terms are similar (Equation 3.16) except that the parasitoid cannibalism vulnerability coefficient, c_P, is used and encounters are between parasitised hosts, P^2, and healthy and parasitised hosts, *HP*.

The zero isoclines for this model are shown in Figure 3.7a and b. Changes in c_H now affect the points where the host isocline crosses both the host *and* parasitoid axes. The isocline crosses the host axis at the same value as in model B for a given value of c_H, and all other parameters the same. This is because at this point there are no parasitised hosts to act as extra cannibals, so the carrying capacity of the host is simply set by the amount of healthy host cannibalism, as in model B. Increasing the value of c_H lowers the point at which the isocline crosses the parasitoid axis, since parasitoids now also limit host increase through cannibalism by parasitised hosts.

The parasitoid isocline crosses the host axis at the same points as in model B, for a given value of c_P, but the shape of the curve is flatter, peaking at lower densities of *P*. The addition of parasitised host cannibalism will obviously have the least effect on the behaviour of the model where there are no parasitised hosts to be cannibals (i.e. where the isocline crosses the host axis). However, the additional parasitoid mortality caused by

parasitised host cannibalism will limit parasitoid increase even further, making the isocline peak at lower values of P than in model B.

The results of combining the two sets of isoclines are shown in Figure 3.7c. Comparing this with the equivalent figure for model B (Figure 3.6c) shows that for given values of c_H and c_P greater than 0, the system reaches equilibrium at a lower density of parasitoids and a higher density of hosts than in model B. Thus, the somewhat surprising result of allowing parasitised hosts to be cannibals is that equilibrium host density *increases* at the expense of parasitoid numbers.

3.4.6 Model D: the components of cannibalism

This final variation of the model considers explicitly the two components of a cannibalistic interaction: the aggression or zeal of the cannibal, and the vulnerability of the victim. The previous models only distinguish between healthy and parasitised hosts in their vulnerability to attack, while when both types cannibalise they do so with equal vigour. However, parasitised and unparasitised hosts may differ not only in their vulnerability to cannibalism, but also in their readiness to attack other individuals. In the model below, the cannibalism vulnerability coefficients for host and parasitoid are c_H and c_P, as before, while z_H and z_P are the respective cannibalism aggressions:

$$X_H = z_H c_H (H+P) H^2 + z_P c_H (H+P) PH$$

Equation 3.17

$$X_P = z_P c_P (H+P) P^2 + z_H c_P (H+P) HP$$

Equation 3.18

The aggression coefficients are expressed relative to the most aggressive class of individual, so, for example, if healthy hosts are more aggressive than parasitised hosts then $z_H = 1$ and $0 < z_P < 1$. If both z_H and z_P are 1 then there is no difference in aggression and the model behaves in the same way as model C, while if they are 0 then there is no cannibalism.

3.4.7 Exploring model D: asymmetries in cannibalism

My analysis of these models has so far concentrated on the effects of general levels of cannibalism on population dynamics. In this section I wish to explore the possible effects that asymmetry in cannibalism ability and vulnerability between the two species might have on their population dynamics. Model D provides the best springboard for this because it allows the full range of possible cannibalistic interactions to be explored. In an extreme case, one species can be made invulnerable to cannibalism ($c_i = 0$) while still being able to attack the other species ($z_j > 0$). Conversely, one species can be vulnerable to attack by the other species ($c_i > 0$), while being unable to retaliate ($z_i = 0$). So for example, if $z_H = 1$ and $z_P = 0$, the model becomes equivalent to model B, where only healthy hosts cannibalised healthy and parasitised hosts.

Figure 3.8 shows how the level of cannibalism aggression affects the equilibrium densities of host and parasitoid. In Figure 3.8a, the healthy host aggression is progressively increased relative to the parasitised host aggression, while cannibalism vulnerability is kept at the same moderate level for both species. The effect of this is to move the equilibrium position towards higher host densities and lower parasitoid densities. Thus the host benefits from increased levels of aggression at the expense of the parasitoid, however, when the parasitoid is absent, the higher cannibalism rates reduce the host's carrying capacity.

Figure 3.8b increases the parasitised host aggression relative to that of healthy hosts. This affects the equilibrium position by once again reducing parasitoid density and increasing host density, while the host-alone carrying capacity remains unaffected this time. These two diagrams show that a host-parasitoid interaction should favour increased aggression in unparasitised hosts while at the same time aggression of parasitised hosts should be reduced.

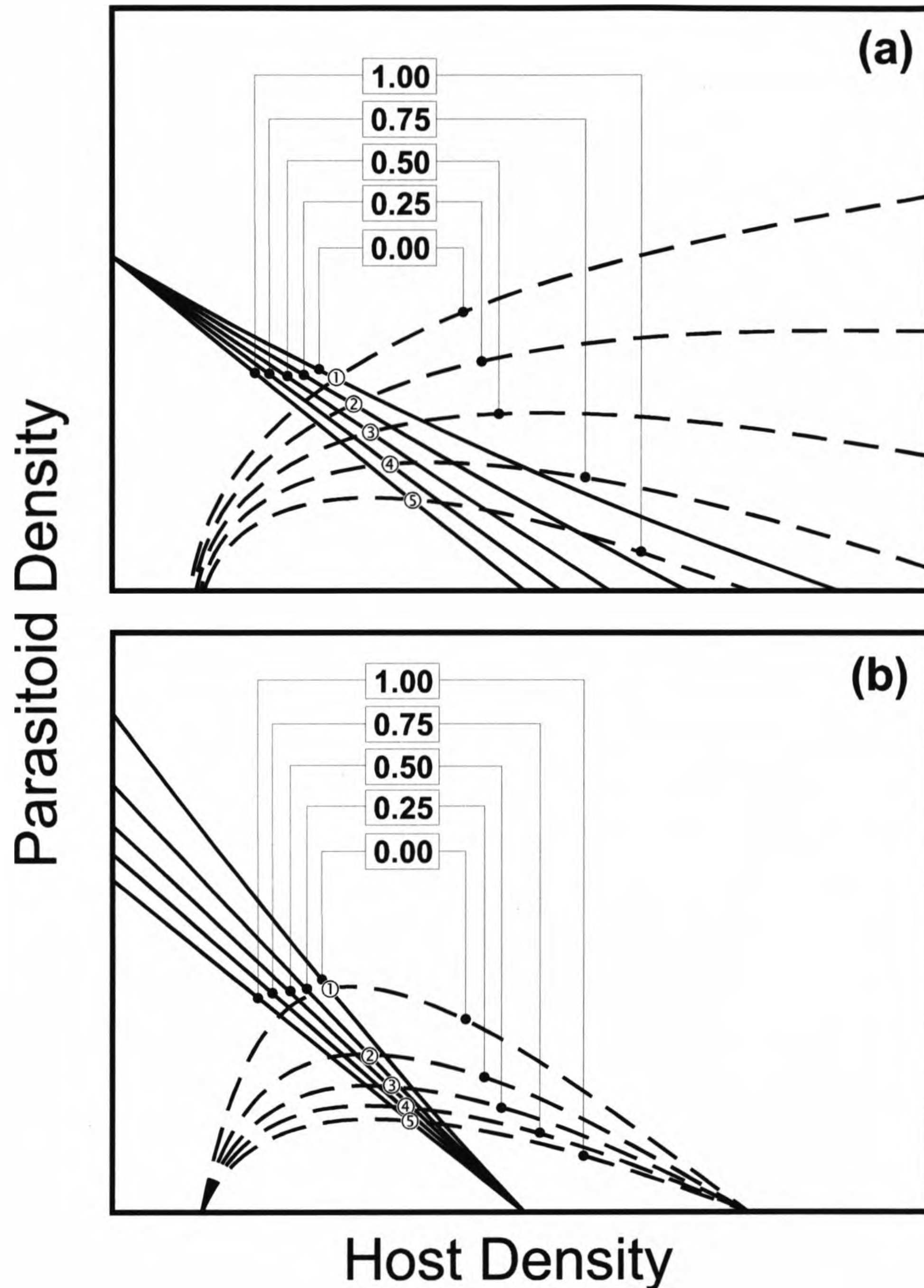

Figure 3.8 Phase plane diagrams for model D, showing the effects of varying cannibalism aggression. In both diagrams, host zero isoclines are shown as solid lines and parasitoid zero isoclines as dashed lines. **(a)** Varying healthy host aggression: $z_P = 1$ while the numbers in boxes are values of z_H. The numbered circles represent stable equilibria for each value of z_H: ① $z_H = 0$; ② $z_H = 0.25$; ③ $z_H = 0.5$; ④ $z_H = 0.75$; ⑤ $z_H = 1$. **(b)** Varying parasitised host aggression: $z_H = 1$ while the numbers in boxes are values of z_P. The numbered circles represent stable equilibria for each value of z_P: ① $z_P = 0$; ② $z_P = 0.25$; ③ $z_P = 0.5$; ④ $z_P = 0.75$; ⑤ $z_P = 1$. Values of other parameters in the model were: $a = 0.4$; $f = 1$; $m = 0.175$; $q = 0.3$; $r = 1.2$; $c_H = 0.1$; $c_P = 0.1$.

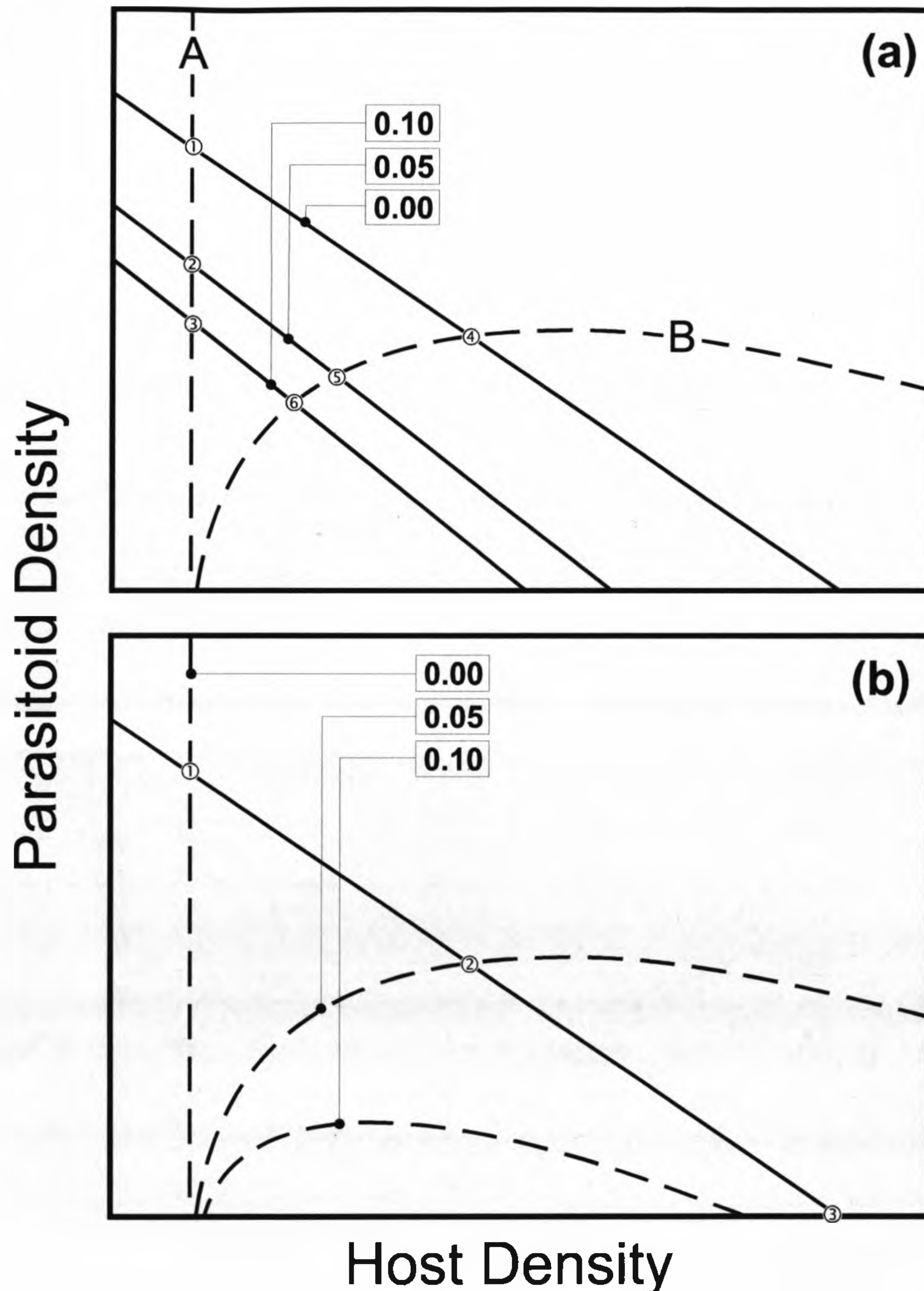

Figure 3.9 Phase plane diagrams for model D, showing the effects of varying cannibalism vulnerability. In both diagrams, host zero isoclines are shown as solid lines and parasitoid zero isoclines as dashed lines. **(a)** Varying healthy host vulnerability with two parasitoid vulnerabilities: A — $c_P = 0$ and B — $c_P = 0.05$, while the numbers in boxes are values of c_H. The numbered circles represent stable equilibria for each value of c_H and c_P: ① to ③ $c_P = 0$; ④ to ⑥ $c_P = 0.1$; ①,④ $c_H = 0$; ②,⑤ $c_H = 0.05$; ③,⑥ $c_H = 0.1$. **(b)** Varying parasitised host vulnerability: $c_H = 0$ while the numbers in boxes are values of c_P. The numbered circles represent stable equilibria for each value of c_P: ① $c_P = 0$; ② $c_P = 0.05$; ③ $c_P = 0.1$. Values of other parameters in the model were: $a = 0.4$; $f = 1$; $m = 0.175$; $q = 0.3$; $r = 1.2$; $z_H = 1$; $z_P = 1$.

The other aspect of cannibalism is the vulnerability of the victims, and this is explored in Figure 3.9. In both diagrams, the parasitised and unparasitised hosts have equal aggressions ($z_H = z_P = 1$), while their vulnerabilities are altered. Figure 3.9a increases host vulnerability from zero at two different parasitised host vulnerabilities. When parasitised hosts are invulnerable ($c_P = 0$), the equilibrium density of parasitoids is reduced while host density remains the same. However, when parasitised hosts are vulnerable ($c_P = 0.05$) the equilibrium parasitoid density is at a lower, but more constant level, while host density also varies. In Figure 3.9b, the healthy hosts are invulnerable to cannibalism, while parasitised hosts are progressively more vulnerable. Once again, the equilibrium parasitoid density is decreased, but now more rapidly, the host density increases, until at high values of c_P, the parasitoid becomes extinct and the host stabilises at its carrying capacity. Therefore, the general effect of increasing healthy or parasitised host cannibalism vulnerability is to reduce the parasitoid equilibrium density.

3.4.8 Summing up: an overview of the effects of cannibalism

The main features of all of these models are:

1. An increase in general levels of cannibalism *always* leads to a decrease in equilibrium parasitoid density and an increase in host density while both species are present.
2. High levels of cannibalism will always lead to extinction of the parasitoid long before the host dies out.
3. Increasing the host's cannibalism vulnerability reduces equilibrium levels of both host and parasitoid. However, increasing parasitoid cannibalism vulnerability still reduces parasitoid density, but host density increases.
4. In the absence of the parasitoid, cannibalism reduces the host's carrying capacity.

Points 1 to 3 illustrate a fundamental asymmetry in a host-parasitoid interaction: the host can survive in the absence of the parasitoid, but the parasitoid is dependent upon the host for survival. Parasitoids need a supply of unparasitised hosts in order to maintain or increase their numbers, so any factor — such as cannibalism — that reduces the equilibrium density of hosts will also reduce the density of parasitoids. However, any factor that depresses only the parasitoids will allow the host density to *increase,* through reduced parasitism.

The direct effect of cannibalism is to reduce the numbers of both hosts and parasitoids, however the indirect effects of cannibalism are different for the two species. A reduction in the parasitoid density through cannibalism will allow the host to increase, because fewer hosts will be parasitised. A reduction in the host density through cannibalism will reduce the number of hosts available for parasitism, so parasitoid density will also be reduced.

3.5 Discussion

These models demonstrate that host cannibalism could have strong effects on the population dynamics of a host-parasitoid system. They may also suggest some interesting possibilities for the evolution of cannibalism in such a system. Although this study is by no means exhaustive it serves as a first step towards understanding the possible role of host cannibalism in host-parasitoid interactions.

The general results of introducing host cannibalism into these host-parasitoid population models are (1) increased stability, (2) a reduction in equilibrium parasitoid densities and usually a corresponding increase in host density, and (3) a smaller region of parameter space where the parasitoid can persist.

The analyses of these models seem to support Hart's (1990) suggestion that cannibalism may sometimes be beneficial to the host of a pathogen or parasite. He argued that if the parasite is not transmitted by cannibalism, then cannibalism of parasitised individuals would benefit the cannibal by removing potential sources of infection. Both general levels of cannibalism and differential cannibalism — which targets only parasitised hosts — lead to suppression of the parasitoid and an increase in host density in these models. However, the mere increase in equilibrium host population levels is not enough to infer an evolutionary advantage from cannibalism. Bobisud (1976) developed a stage specific model incorporating prey cannibalism in a predator-prey interaction, in which cannibalism allowed the prey to escape the vulnerable stage more quickly. He found that prey cannibalism could increase the equilibrium population density of prey, and argued that this would lead to a selection pressure for the evolution of cannibalism. However, his argument was later criticised (Stenseth and Reed, 1978) for relying on group selection principles rather than examining the fitness of individuals. An individual that cannibalises

in the presence of parasitoids will increase its fitness through reduced parasitism of its offspring. However, in the absence of the parasitoid the host's carrying capacity is reduced by cannibalism, so here is a situation where cannibalism will only be selected for in the presence of a parasite.

The models that I have developed here are relatively simple, and limited in their scope. There are a number of ways in which cannibalism could be explored more realistically, but these methods would add complexity and make analysis less tractable. One approach would be to model the cannibalistic stage more explicitly. Cannibalism usually occurs at a specific stage in a life-cycle, and the victims of cannibalism can either be the same or a different stage. The two-equation models in this chapter encompass all stages of each species within a single equation, but a more explicit approach would be to use separate equations for the cannibal, victim, and invulnerable classes of each species. The drawback of this approach is that the flow of individuals between the various classes would have to be modelled using complex delay-differential terms which would increase the parameter count and lose the analytical power of simpler models. Perhaps a better method would be the individual-based foraging approach suggested by Dong and Polis (1992) for while such models have no analytical solution they are probably better able to cope with the complex dynamic interactions involved in cannibalism.

4. Density-dependence in cohorts of *Plodia interpunctella*.

4.1 Introduction

The density at which an animal is reared can have a wide range of effects on its growth, reproductive potential and behaviour. An understanding of these density-dependent effects is essential for developing accurate models of population dynamics. In this and the following chapter I describe two experiments designed to explore some of the effects of initial density on cohorts of *P. interpunctella* as they develop from eggs to adults.

4.1.1 What is density?

In its simplest form, density is the number of animals per unit of resource (Begon *et al.*, 1990), yet this simple definition hides many subtleties. The resource against which density is measured may be space (area or volume), food, pupation sites or any other factor which is in limited supply. The response of animals to density may be scale-dependent — 1 individual in 1 m^2 of habitat may behave differently to 100 individuals in 100 m^2 of habitat, even though they are at the same density of 1 individual per m^2 (Wiens, 1989; Smallwood and Schonewald, 1998). At smaller scales, absolute numbers of individuals or amounts of resource may be more important than simple density (Smallwood and Schonewald, 1998). Density is not static within a particular system, but will vary as resources are used up or renewed, and as animal numbers fluctuate due to birth, death, emigration and immigration.

4.1.2 Constraints and trade-offs

Animals are not infinitely variable and plastic in their responses to different conditions. There are constraints on how far they can optimise their response to different levels of resource (Tammaru, 1998). Different aspects of an animal's fitness may be limited by density in different ways, and this will force a trade-off in the ratio of resources devoted to different body functions (Guntrip *et al.*, 1997). At least part of these trade-offs will be genetically determined (Møller *et al.*, 1989a), but there may also be scope for an animal to vary the trade-off components in response to density (Møller *et al.*, 1989b).

4.1.3 Density and growth

The most immediate effect of higher densities is usually to limit food supply. Competition between individuals for a finite amount of food will mean that as density increases, each individual animal will get less food. This will affect an animal's ability to develop and grow, while in extreme cases it may not even get enough food to maintain its current body functions and it will die. Growth responses to density may be in the rate of growth (Wissinger, 1988) or eventual size (Baur, 1988; Giga and Smith, 1991) or a combination of both. Even if an animal survives to adulthood, high density during development may reduce the storage of nutrients and so adult lifespan is shortened (Parajulee and Phillips, 1995).

4.1.4 Density and reproduction

As an animal's growth becomes limited by density, it faces a trade-off between investment in reproductive and somatic tissues that will ultimately affect its reproductive success and fitness (Holloway *et al.*, 1987; Rees and Crawley, 1989). Investment in reproduction will be of no use if the animal does not survive long enough to reproduce, so it has to achieve the optimum balance between survival and reproduction. Some of this balance may be genetically determined (Brough and Dixon, 1989; Møller *et al.*, 1989a; Guntrip *et al.*, 1996), but it can also be varied by individuals in response to density (Gage, 1995; He and Miyata, 1997). For females, the end result will be density-dependent variation in clutch-size (Brunsting and Heessen, 1984; Baur, 1988), or a trade-off between clutch size and egg size, which can affect the subsequent development of the offspring (Guntrip *et al.*, 1997).

Another effect of density on reproduction may be in the expectation of competition for mating opportunities. This may not affect females, whose reproductive success is mainly limited by the number of eggs that they can produce, but male reproductive success depends on successful fertilisations. Higher densities increase the competition for mates, and the likelihood of sperm competition, so males may adjust their reproductive strategy according to density (Gage, 1995; He and Miyata, 1997).

4.1.5 Density and behaviour

Another way in which an animal can respond to density is through behaviour. It may try to escape high densities by emigrating to other areas (Islam *et al.*, 1994), or it may try to gain a larger share of the available resources by aggression or cannibalism. Adults may also

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attempt to place their offspring in areas where they are likely to suffer less competition (Anderson and Löfqvist, 1996). Cannibalism is discussed in greater detail in chapters 2 and 3, but is worth mentioning here as an important behavioural response to density. Cannibalism is sometimes called a "life-boat" strategy (Cushing, 1992) — a last-ditch response to extreme density. In most situations, cannibalism is risky due to possible injury (Harris, 1989), or in terms of disease transmission (Schaub *et al.*, 1989; Pfennig *et al.*, 1991; Boots, 1998).

4.1.6 Density-dependence in *P. interpunctella*

Several studies have already looked at density effects in *P. interpunctella*. Snyman (1949) found that higher densities during development led to smaller adult size and reduced survival. There was a strong size-fecundity relationship for females, and higher adult densities reduced the number of eggs laid by each moth. Density effects on size and mortality, and the same size-fecundity relationship were found also by Podoler (1974b). He demonstrated a threshold between density-independent and density-dependent larval mortality as density increased. A more recent study (Anderson and Löfqvist, 1996) found no effect of density on adult size (though see the discussion of this chapter), but did find that females preferred to lay their eggs in food with low densities of larvae already present. Gage (1995) found that at higher densities (different numbers per unit volume, same *per capita* food), adult males had relatively larger testes, ejaculate more sperm, and had shorter lives.

The experiment described in this chapter was intended to explore how different initial numbers and densities with food affected the egg-to-adult survival of cohorts of *P. interpunctella*. It also looked at the effects of larval density on adult size and the reproductive potential of both males and females.

4.2 Method

4.2.1 Introduction

This experiment was to examine the fate of cohorts of *P. interpunctella* eggs as they developed to adulthood at different densities. The basic design was a full factorial experiment, with food amount and initial egg number as factors. Survivors to adulthood were counted, and a subset of these survivors were dissected to examine possible effects

of density on reproductive potential. I collaborated with Dr S. Sait in the planning and running of this experiment, however the data analysis and interpretation are my own.

4.2.2 Set up

The 28°C cultures (see section 1.2.3) were used for this experiment. Newly emerged adult moths (<24 hours old) were placed into smaller versions of the egg machine described in chapter 2, and eggs were collected over a 24 hour period. The experiment was set up in 4 blocks, with a different batch of eggs (from different moths) being used for each block. One hundred eggs from each batch were tested for egg hatch using the method described in section 1.2.5.

The experiment was a full factorial design, with 6 different food weights — 0.25, 0.5, 1, 2, 4, and 8g — and 6 different egg numbers — 1, 2, 4, 8, 16, and 32 eggs. The 36 treatments consisted of each possible food weight – egg number combination. The experiment was set up in 4 blocks, with three replicates of each treatment in each block. The set up order of treatments and replicates was randomised within each block. The food used in the experiment was the same as that used for the cultures except that it had been ground finely in a coffee grinder to make measurement easier. Food was weighed into 30 ml universal tubes using a top-pan balance, then eggs were added using a fine brush. The lids of the tubes were screwed on slightly loose to allow air to circulate, and the tubes were laid on their sides in trays in an incubator.

4.2.3 Monitoring

The tubes were checked 1 to 3 times each day while moths were emerging in them. Newly emerged moths were removed and placed individually into numbered Eppendorf tubes, before being frozen. The replicates were checked more often at times of peak emergence to try and ensure that most of the moths were virgins — i.e. that they were alone in a tube when they were collected. Nonetheless, some tubes did contain more than one moth at a particular check, and these moths were considered to be non-virgins for the measurements and analysis that followed. Monitoring did not continue past day 48 for each block, since moths that emerged after this time could conceivably have been second generation offspring of the first emergers.

4.2.4 Measurements

Several measurements were taken from the frozen dead adults:

1. Up to 10 females from each treatment were randomly selected for dissection. The ovaries (without the bursa or accessory glands) were removed and dry weighed. Both virgin and non-virgin females were dissected, since the ovary weight would not be affected by mating status. Also, females usually do not start to lay eggs until a day or so after mating (see chapter 6), so even mated females would not have laid any eggs before collection.
2. Up to 10 males from each treatment were randomly selected for dissection. The abdomens were dissected open, and their testes were measured using a microscope with an eyepiece graticule. The males had a single fused testis, which was roughly a "flattened" sphere in shape, so both the maximum and minimum diameter were measured. The testis was a very flexible organ, whose size was greatly affected by whether or not the males had mated (M. Gage, pers. comm.), therefore only known virgin males were dissected.
3. If there were any moths remaining in a treatment, up to 30 of each sex were randomly selected, and their whole bodies dry weighed. Both virgins and non-virgins were weighed, since the small size of an ejaculate would probably have had a negligible effect on the dry weight of the whole body.
4. Right mid femur lengths were measured on all of the moths that were dry weighed or dissected. The mid femur was chosen as a linear measure of body size because it lay flat and had well-defined defined ends when the leg had been pulled off the body, making it easily measurable using a microscope eyepiece graticule. Other leg segments were either curved or had tufts of scales which made consistent measurement difficult. Wing length could not be used as measure of body size, since many of the moths were newly emerged and had not fully expanded their wings.

Dry weights were taken by placing the bodies or ovaries into pre-weighed foil weighing boats, before drying in an oven at 60°C for at least 4 days. The weighing boats with contents were then re-weighed using a Cahn electrobalance.

4.3 Results

4.3.1 General information

Twenty of the 432 replicates in the experiment produced more adult moths than the number of eggs they were supposed to contain. These were assumed to be set up errors

or contamination and were excluded from any analyses (see Table 4.2). Other replicates produced no adults at all, but this may have been due to mortality rather than set up errors or escapes, and so these tubes were included in analyses where appropriate.

		Food					
		0.25g	0.5g	1g	2g	4g	8g
Eggs	1	**12**(10)	**11**(7)	**9**(8)	**11**(11)	**10**(7)	**11**(5)
	2	**12**(9)	**11**(10)	**12**(9)	**11**(8)	**11**(6)	**10**(7)
	4	**12**(10)	**12**(10)	**11**(9)	**12**(11)	**12**(8)	**12**(8)
	8	**12**(10)	**12**(10)	**10**(9)	**11**(10)	**11**(10)	**10**(10)
	16	**12**(2)	**12**(9)	**12**(9)	**12**(9)	**12**(7)	**12**(11)
	32	**12**(0)	**12**(4)	**12**(10)	**12**(11)	**12**(9)	**12**(11)

Table 4.2 Analysed replicate numbers for each treatment. Figures in brackets are the number of replicates in which any moths emerged.

The mean egg hatch rate for the whole experiment was 0.87. Egg hatch varied between blocks, but the differences were not significant ($\chi^2 = 1.368$, d.f. = 3, $p > 0.05$). The mean egg hatch rate was used to calculate initial larval numbers and density values for the rest of the analysis as follows:

Initial larval number = initial egg number × egg hatch;

Density = (initial egg number × egg hatch) / food weight.

Steps were taken to avoid pseudoreplication in analysis of the data. The design of the experiment replicated each food – egg number combination several times, and several moths emerged from each tube in most of the treatments. The competitive conditions within a tube would have depended on mortality and individual differences between competitors. Within a treatment, these factors would have varied between tubes in a reasonably random and independent manner, and thus each tube could be considered a true replicate. The moths emerging from a single tube had all experienced the same conditions and so were *not* independent of each other. Using the values from individual moths in analysis of treatment effects would have led to pseudoreplication and overestimates of sample sizes. To avoid this, mean values of the characteristics of moths from each tube were used in analyses of treatment effects. Analyses of general relationships between size measurements were done across all treatments and so were not subject to pseudoreplication. These analyses used the data on individual moths.

4.3.2 Measures of size

The right mid femur length was measured on all of the moths examined in the experiment. Once a moth had been dissected it was impossible to retrieve all of the tissues for a whole body dry weight, so it was hoped that the femur length would provide a link between the ovary and testis dissections and the whole body dry weights. Figure 4.1 shows that a power function provided a good fit between right mid femur length and dry body weight. If dry body weight was directly proportional to volume, then one would expect weight to be proportional to $length^3$. The exponents in the regressions for males and females were significantly different from each other, but both were also significantly greater than 3, implying that dry body tissue density increased with increasing linear body size. This was supported by the discovery of large fat deposits in the larger moths that we dissected. These fat deposits would not have been reduced in weight as much as other tissues during drying, and this could have led to the observed weight-length relationship.

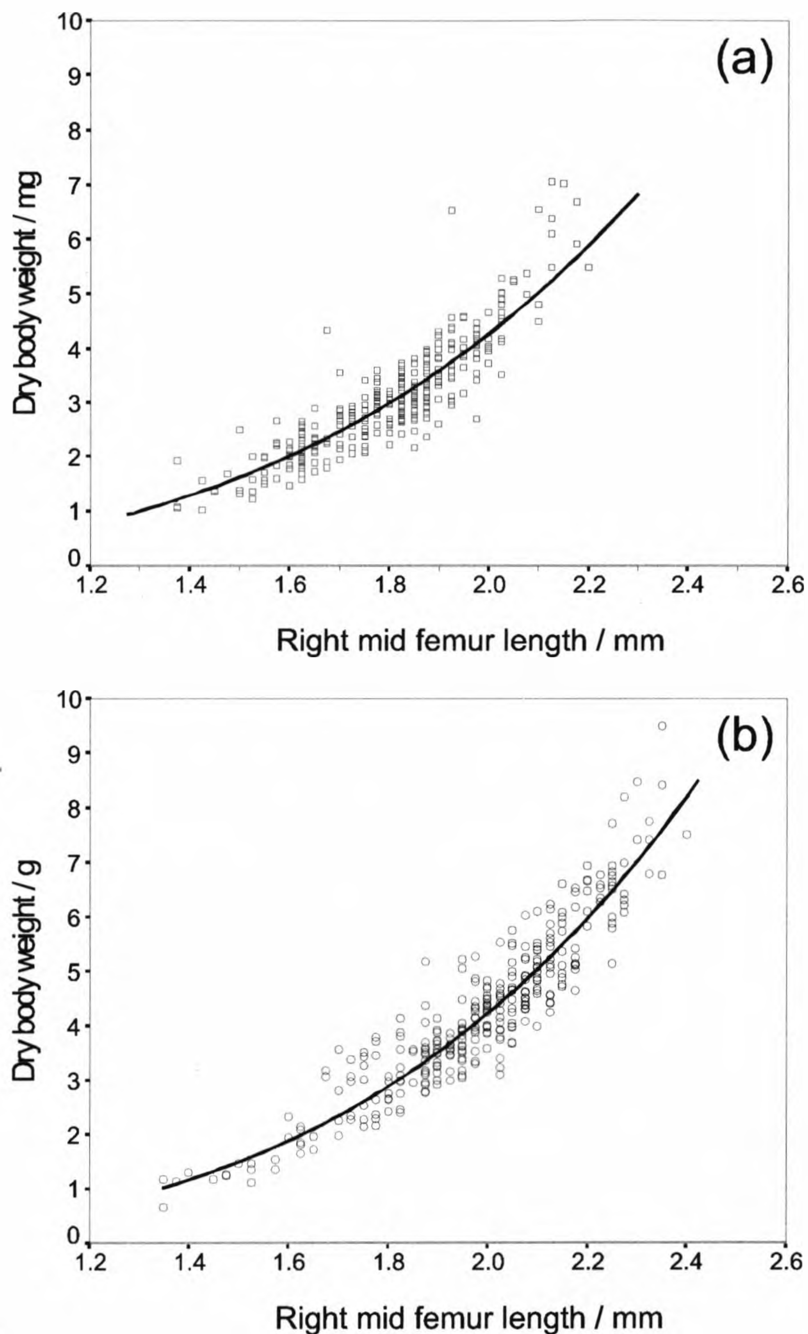


Figure 4.1 The relationship between dry body weight and right mid femur length (RMFL). **(a)** males, regression line: dry body weight = 0.41 $RMFL^{3.38}$, $r^2 = 0.803$, d.f. = 305, $p < 0.001$; **(b)** females, regression line: dry body weight = 0.34 $RMFL^{3.63}$, $r^2 = 0.881$, d.f. = 332, $p < 0.001$. The regression lines are significantly different from each other ($t = 3.23$, d.f. = 607, $p < 0.01$), and are significantly different from a dry body weight = $RMFL^3$ relationship for both males ($t = 3.94$, d.f. = 305, $p < 0.001$) and females ($t = 8.59$, d.f. = 332, $p < 0.001$).

4.3.3 Ovary weight

A regression of ovary weight against femur length found that a power function best described the relationship (Figure 4.2). The exponent was significantly greater than that for the relationship between female whole body dry weight and femur length ($t = -7.02$, $d.f. = 595$, $p<0.001$), and this implied that larger moths had proportionally larger ovaries.

4.3.4 Testis volume

The testis in *P. interpunctella* is shaped like a "flattened" sphere, and the minimum and maximum diameters of the testis were measured. To approximate the volume of the testis, Gage (1995) averaged these two measurements and used this average as the diameter of a sphere. However, the volume of this shape is more accurately described by the formula:

$$Volume = r_{\max}{}^{2} r_{\min} \frac{4}{3}\pi ,$$

and this was the formula that I used for calculating testis volume in this analysis.

Figure 4.3 shows that, once again, a power function provided the best fit for testis volume against femur length. However, the exponent in the regression was not significantly different from that for male body weight ($t = 1.04$, $d.f. = 570$, $p>0.2$). Therefore, unlike ovary weight, testis volume scaled in direct proportion to body size.

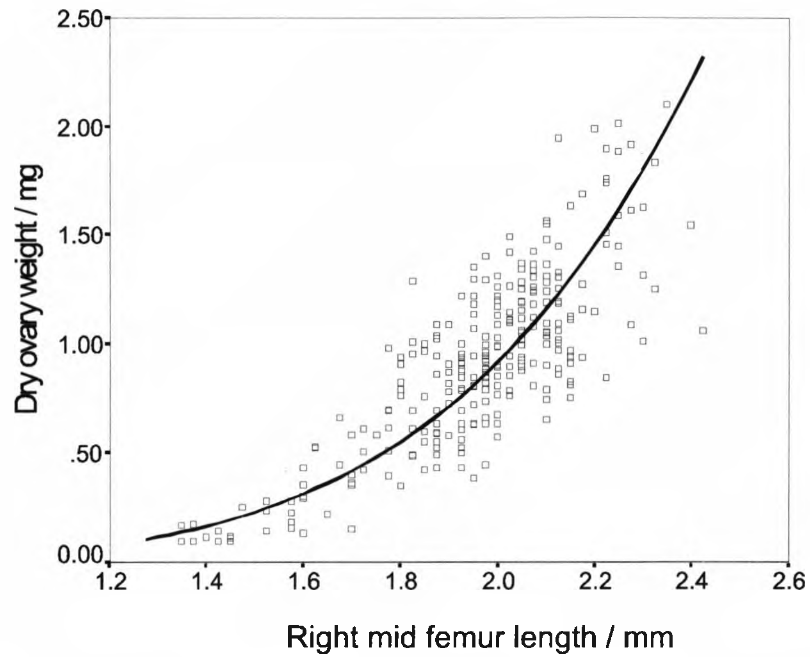


Figure 4.2 The relationship between ovary dry weight and right mid femur length. Regression line equation: ovary weight = 0.03 right mid femur length$^{4.84}$, $r^2 = 0.766$, d.f. = 263, $p < 0.001$.

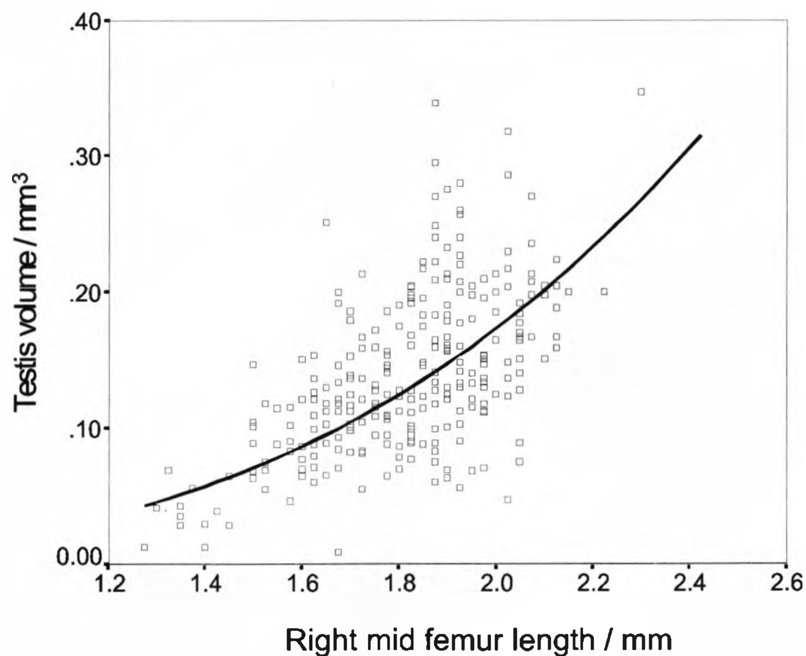


Figure 4.3 The relationship between testis volume and right mid femur length. Regression line equation: testis volume = 0.02 right mid femur length$^{3.11}$, $r^2 = 0.395$, d.f. = 265, $p < 0.001$.

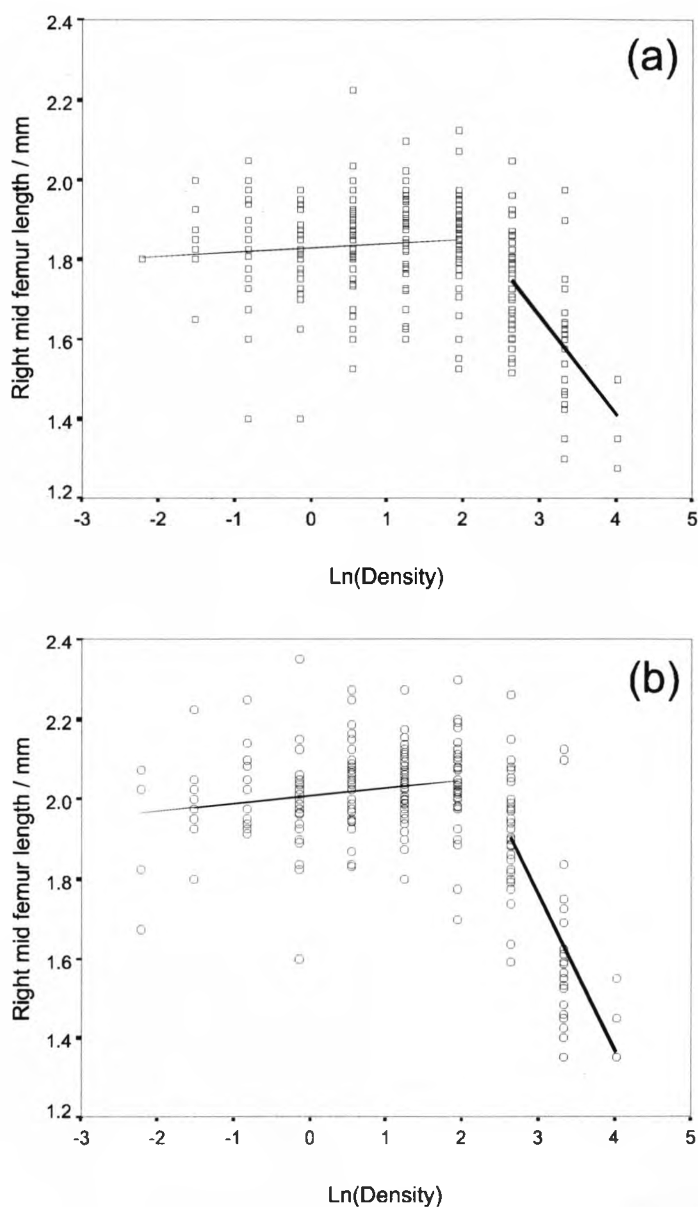
4.3.5 Size and density

The relationship between femur length and density showed a clear threshold between density-dependent and density-independent regions (Figure 4.4). For densities greater than about 9 larvae per g, femur length decreased with increasing density, while below this threshold, there was no effect (for males), or only a weak effect (for females) of density on femur length. The very low r^2 value (0.036) for the low density regression in females suggested that although the regression was statistically significant, the true effect of density on body size was not important at low densities.

Dry body weight scaled with femur length (see section 4.3.2), and so density could affect body weight through changes in femur length. There may also have been additional effects of density on body weight, over and above those explained by femur length. To test this, I first transformed femur lengths using the exponential relationships found in section 4.3.2, to generate a linear relationship between body weight and femur length. I then used stepwise regression of body weight against density *and* transformed femur length, to see if the addition of density explained any more of the variance in body weight than femur length alone. However, density was not entered (p-to-enter ≤ 0.05) in these regressions for either males or females, so there were no significant extra effects of density on body weight.

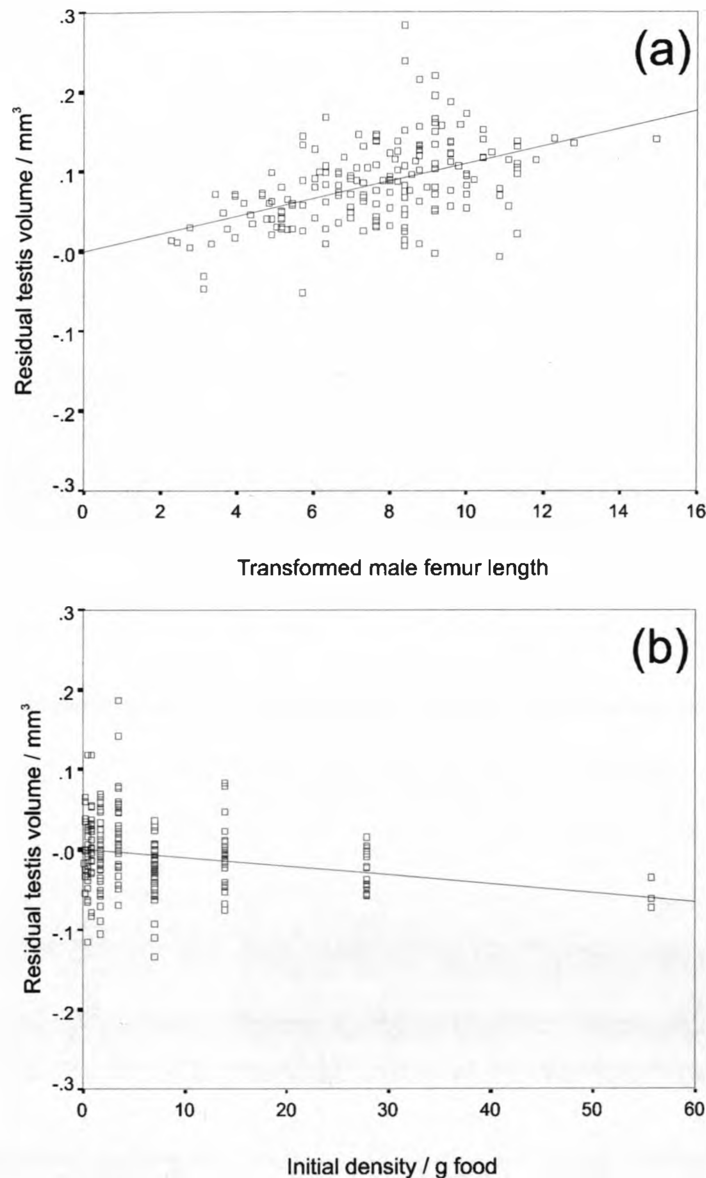
4.3.6 Density and reproductive investment

Reproductive investment — in terms of ovary or testis size — may have changed in response to density. However, ovary and testis size were strongly dependent on femur length (see Figure 4.2 and Figure 4.3), which was, in turn, dependent on density (see Figure 4.4). Once again, to untangle the effects of body size and density on ovary and testis size, I transformed the femur lengths by the relationships in Figure 4.2 and Figure 4.3, and then used stepwise regression of ovary weight and testis volume against transformed femur length and density. This time, density was entered into the model for both testis volume and ovary weight. Figure 4.5 shows the results of the regression for testis volume, and Figure 4.6 shows the results for ovary dry weight. In both cases, density had a significant, negative effect on reproductive investment, however the strengths of the density effects, and the extra variance explained by adding density were very small.



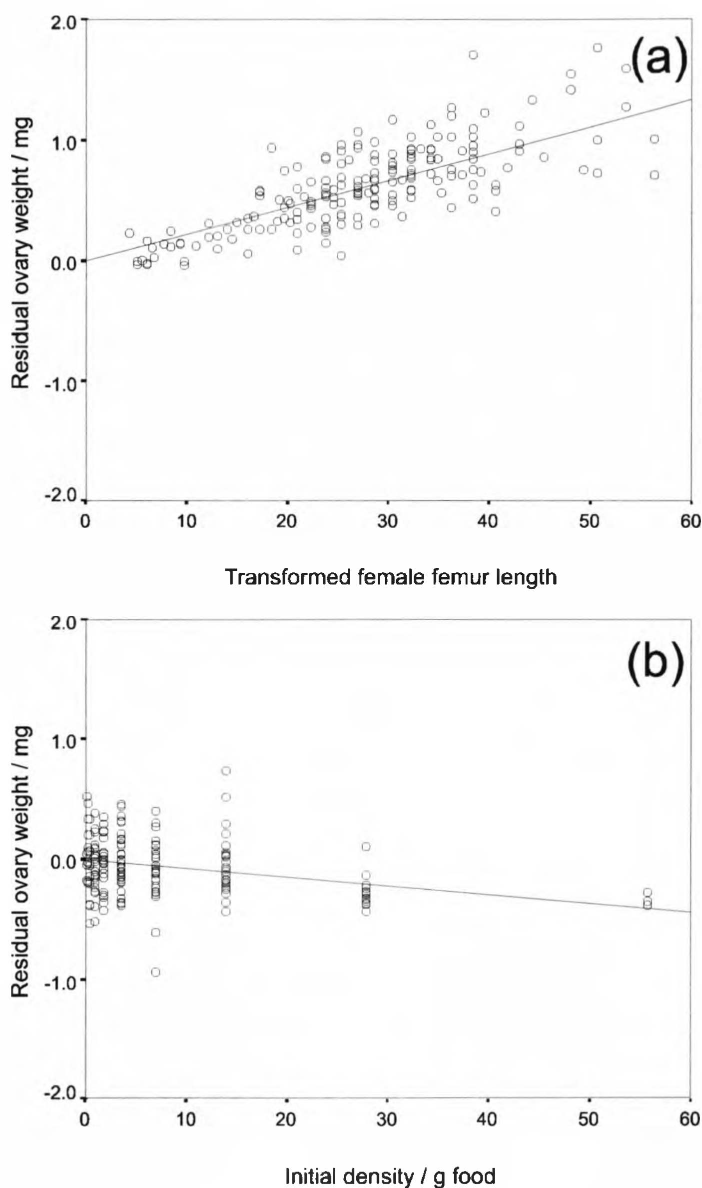
	Ln(Density)	Equation	r^2	d.f.	p
(a) Males	Low	RMFL = 1.83 + 0.01 Ln(Density)	0.008	176	0.232
	High	RMFL = 2.39 - 0.25 Ln(Density)	0.351	59	<0.001
(b) Females	Low	RMFL = 2.01 + 0.02 Ln(Density)	0.036	172	0.012
	High	RMFL = 2.93 - 0.39 Ln(Density)	0.492	61	<0.001

Figure 4.4 Non-linear regressions of right mid femur length (RMFL) against ln(density). The regression fitted two different straight lines to the data, for ln(density) values greater than (heavy line) and less than or equal to 2 (thin line). Choosing this value maximised r^2. In males the high and low density lines cross at ln(density) = 2.15 (density = 8.6 larvae/g), while for females they cross at ln(density) = 2.24 (density = 9.4 larvae/g). The slopes of the high density regression lines for males and females were not significantly different (t = 0.98, d.f. = 120, p > 0.2).



Model	Parameters	Coefficients	p	r^2
1	Constant Transformed femur length	0.035 0.013	0.004 <0.001	0.294
2	Constant Transformed femur length Density	0.061 0.011 -0.001	<0.001 <0.001 0.004	0.325

Figure 4.5 Stepwise multiple regression of testis volume against transformed femur length and density. The table shows the results of regressions without density (**Model 1**) and with density (**Model 2**) entered as a parameter. Femur length was transformed to linearise its relationship with testis volume (see text for details). The graphs show the partial residual testis volumes from model 2 plotted against **(a)** transformed femur length and **(b)** density.



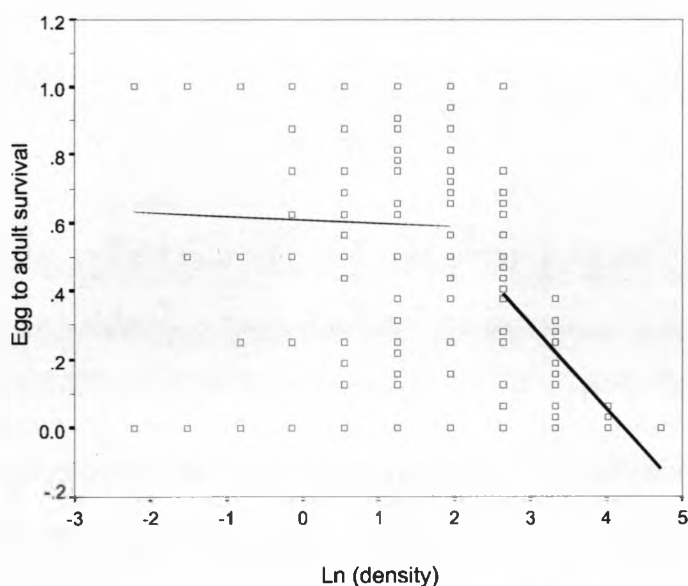
Model	Parameters	Coefficients	p	r^2
1	Constant	0.207	<0.001	0.605
	Transformed femur length	0.025	<0.001	
2	Constant	0.354	<0.001	0.634
	Transformed femur length	0.022	<0.001	
	Density	-0.007	<0.001	

Figure 4.6 Stepwise multiple regression of ovary weight against transformed femur length and density. The table shows the results of regressions without density (**Model 1**) and with density (**Model 2**) entered as a parameter. Femur length was transformed to linearise its relationship with ovary weight (see text for details). The graphs show the partial residual ovary weights from model 2 plotted against **(a)** transformed femur length and **(b)** density.

4.3.7 Survival and density

Density had significant effects on egg to adult survival. To avoid obtaining survival values greater than one, I have used initial egg numbers in this part of the analysis rather than the estimated initial larval numbers used elsewhere. Thus, the survival rates implicitly include the mean egg hatch rate of 0.87. Density values were still calculated using estimated initial numbers of larvae, since the larvae were the stage that was most likely to be density-sensitive.

Figure 4.7 shows the relationship between survival and ln(density). Note that survival seemed to be split into density independent and density dependent regions as density increased. Below ln(density) = 2, there was no significant effect of density on survival, while above this threshold, density had a strong, negative effect on survival. This was a similar response to that of body size to density (see Figure 4.4).



Ln(Density)	Equation	r^2	d.f.	p
Low	Survival = 0.609 - 0.01 Ln(Density)	0.001	290	0.601
High	Survival = 2.39 - 0.25 Ln(Density)	0.351	118	<0.001

Figure 4.7 Non-linear regression of egg to adult survival against ln(density). The regression fitted two different straight lines to the data, for ln(density) values greater than (heavy line) and less than or equal to 2 (thin line). Choosing this value maximised r^2. The high and low density lines cross at ln(density) = 1.82 (density = 6.19 larvae/g).

4.3.8 Density and development time

Density had a significant effect on egg-to-adult development time (Figure 4.8). There was no obvious threshold between density-dependent and density-independent responses, unlike survival and body size.

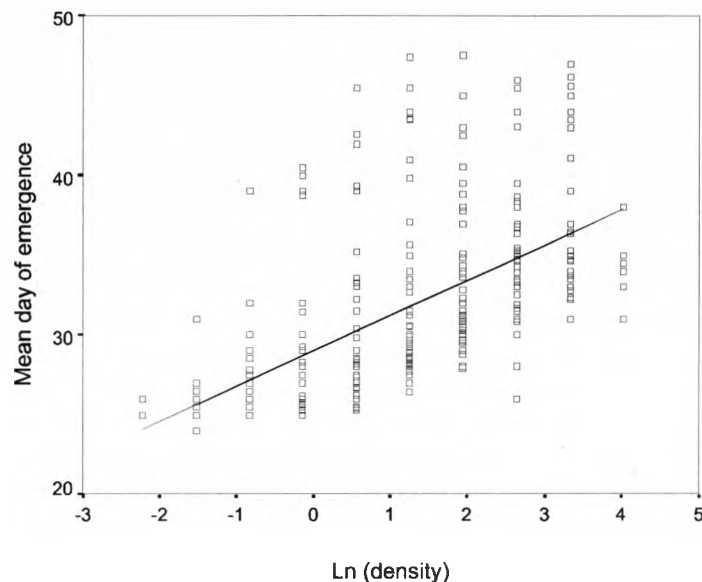


Figure 4.8 Development time against ln(density). The formula for the regression line was: Mean day of emergence (days after egg-laying) = 29.02 + 2.21 Ln(density); r^2 = 0.328, d.f. = 302, $p < 0.001$.

4.4 Discussion

Murray (1982) defines a density-dependent factor as "any component of the environment whose intensity is correlated with population density and whose action affects survival and reproduction". This study demonstrates that density can affect survival and reproduction, but it also highlights the complexities of these density-dependent responses: there may be thresholds below which density has no effect. Previous studies of density effects in *P. interpunctella* (Snyman, 1949; Podoler, 1974b) have also found thresholds in the effects of density on survival. Podoler (1974b) demonstrated a threshold density of around 14 larvae per g food after which mortality increased rapidly, while the threshold density in Snyman's (1949) experiment was somewhere between 7.5 and 5 larvae per g food. This difference was explained by Podoler in terms of the higher quality food that he used. However, his food recipe was very similar to the one that I used in my

experiment, and yet the threshold of around 9 larvae per g that I found in my results fell somewhere between those of Snyman and Podoler.

A possible reason for this paradox may have been the conditions under which the experiment was run. Temperature may have had an effect: Podoler used a temperature of 30°C, while my experiments were run at 28°C. A more likely explanation, however, was that Podoler included a strip of corrugated paper to provide pupation sites, while in my experiment there was none. The experiment in Chapter 5 demonstrated that pupal cannibalism was a significant source of mortality at high densities, and Podoler's strips of corrugated paper may have provided the pupae with a form of refuge. This refuge would have delayed the onset of density-dependent mortality, so raising the threshold that he found relative to mine.

My study also found a threshold in the effect of density on size (as measured by femur length), at the same point of 9 larvae per g. There is no evidence of a similar threshold in either Podoler's (1974b) or Snyman's (1949) data, although both show strong negative effects of density on body size. However, both studies agree with mine in showing a stronger effect of density on females than on males — density reduced and eventually removed the normal size dimorphism between the two sexes. Anderson and Löfqvist's (1996) study found no effect of density on adult weight, but they only used three different densities, and their maximum density was 10 larvae per g food, so any density effect may not have become apparent. They also used a different food type, and do not say at what temperature they ran their experiments, so it may not be possible to compare directly their data with the experiment in this chapter.

These thresholds in the response of size and survival to density strongly suggest that the type of competition found in *P. interpunctella* is closest to 'scramble' rather than 'contest'. In contest competition some individuals are assumed to have a competitive advantage over others, so in situations where food is limiting these better competitors will always be more likely to survive. Under pure contest conditions, on a given amount of food, one would expect a constant number of survivors regardless of the starting number — also called 'exact compensation'. In scramble competition, all individuals have the same competitive ability so, as density increases, each individual receives less food. At extreme densities there is not enough *per capita* food for *any* individual to survive — 'overcompensating' mortality. Begon, Harper and Townsend (1990) use Snyman's (1949)

data on *P. interpunctella* as an example of 'scramble-*like*' competition where there is "considerable, but not total overcompensating mortality". However, my experiments in this chapter and the next showed that, at high enough densities, *all* of the individuals died, so *P. interpunctella* is an example of 'pure' scramble competition with completely overcompensating mortality.

Animals with limited resources face a trade-off in how they allocate those resources to reproduction and survival in order to maximise their fitness. Theory suggests that in short-lived, semelparous insects like *P. interpunctella*, female reproductive mass (≈ ovary weight) should scale with body weight$^{1.00}$ (Holloway *et al.*, 1987). Although body weight and ovary weight were not measured in the same individuals in this experiment, there were strongly significant relationships between body weight and femur length$^{3.63}$ and between ovary weight and femur length$^{4.84}$. If femur length can be used to link ovary weight to body size, this makes ovary weight proportional to body weight$^{1.33}$, which is a higher scaling factor than predicted by theory.

This high scaling factor was probably caused by a reproductive threshold acting on the smallest moths. At the lowest body sizes, ovaries were extremely small — with perhaps less than ten well formed ovarioles — and only occupied a small proportion of the space in the abdomen. In larger moths, the ovaries almost filled the abdomen — with hundreds of ovarioles — and most of the remaining space was filled with fat storage. The smallest females were from the highest densities, and were probably close to a reproductive threshold (Rees and Crawley, 1989). They needed to devote all of their resources to somatic tissues simply to stay alive, with little or none left for reproduction. There was simply no choice for these moths in the trade-off between reproduction and survival. This was supported by results from the experiment described in the next chapter which showed that moths from higher densities had shorter adult life-spans (see section 5.3.7). Thus, the predicted reproductive mass – body weight relationship may break down when animals are at extreme body sizes.

Density can affect reproductive investment in two ways: firstly it can affect body size, which in turn affects reproductive investment (see above), and secondly density can affect reproductive investment directly through changes in the ratio of resources devoted to reproductive or somatic tissues. These direct effects of density are harder to pick out, since the effects of body size have to be eliminated first. Once size was controlled for,

density did have significant negative effects on reproductive investment in both males and females, yet the effects were extremely weak. Gage (1995) found that *P. interpunctella* males adjusted the size of their testes in response to density, with males reared at higher densities having larger testes relative to body size. He suggested that males were adjusting ejaculate sizes in response to their expected levels of sperm competition. My experiment found the opposite effect, with males reared at higher densities having slightly *smaller* testes relative to body size. The reason for this difference is unclear, but it may partly be due to differences in experimental set up. Gage's experiment created different 'densities' by having different numbers of larvae in a container, but with the same *per capita* food amount, while in my experiment *per capita* food varied. In Gage's experiment, treatments would have varied in the encounter rates between individuals without having differences in food stress. In my experiment, males may have been limited in their ability to adjust testis size by food stress, and so the effect found by Gage was absent or obscured.

Although all of the effects of density that I have discussed here are on adult moths, these effects are really only the consequence of *larval* density. By the time a larva pupates, the die is pretty well cast in terms of the characteristics of the adult that will emerge. The next chapter moves on to examine the development of the density-sensitive stage — the larvae — and how this might relate to the characteristics seen in the adults.

5. Stage-frequency analysis of *Plodia interpunctella* cohorts.

5.1 Introduction

The development of insects is not a continuous process, but rather is punctuated by a series of major and minor changes: eggs hatch into larvae which go through a series of moults (instars) as they grow, then emerge, with or without a pupal stage, as adults. Thus it is often more appropriate to consider the development of insects in terms of these life-stages, rather than purely in terms of age (Manly, 1990).

A consideration of the interactions within and between different stages is important for realistic mathematical models of insect population dynamics (Loreau, 1990; Nisbet and Onyiah, 1994). Using data, such as survival and development times, from experiments that rear individuals in isolation may not provide an accurate picture of what happens when groups of individuals are reared together. Long-term data sets of *P. interpunctella* populations have been collected by previous workers at Liverpool (Sait *et al.*, 1994; Begon *et al.*, 1996; Sait *et al.*, 1998), and there is now much interest in modelling these data-sets (Bjørnstad *et al.*, 1998; Briggs *et al.*, *in prep.*) using stage-structured techniques. This has prompted the need for better estimates of the overall and stage-specific life-history parameters that are used in the models, and the work described in this chapter and the previous one was aimed at providing some of these estimates.

5.2 Method

5.2.1 Introduction

This experiment was designed to observe the development and survival rates of single cohorts of *P. interpunctella* larvae at different initial densities. The basic method was to set up 4 different densities of eggs in a small amount of food, then once the eggs had hatched, the food was searched each day, and the numbers of each instar counted. I collaborated with Dr S. Sait in the planning and running of this experiment, however the data analysis and interpretation are my own.

5.2.2 Set-up

The animals used for the experiment came from the 28°C cultures, and the experiment was run in the same conditions as for these cultures (see section 1.2.3). Newly emerged (< 24 hours old) moths were taken from the cultures and placed into smaller versions of the egg machine described in chapter 1. Eggs were collected after 24 hours and counted into 60ml plastic pots with lids, containing 0.5g of food. The day on which the moths were placed in the egg machines was counted as day 0, therefore the treatments were set up on day 1. The food had been ground finely in a coffee grinder to make it easier to search through, but it was otherwise the same as the normal food for the 28°C cultures.

Four, 8, 12 or 16 eggs were counted into each pot to generate 4 different treatments with 20 replicates of each treatment. These densities were chosen in the light of a pilot experiment, and the experiment described in chapter 4, to cover the threshold between density-independent and density-dependent behaviour. Ten replicates of each treatment were set aside as undisturbed controls, to determine whether repeated searching of the experimental treatments affected survival. The remaining replicates were used for the experimental treatments. All of the replicates and treatments were set up on the same day from the same batch of eggs, and were set up in a random order. One hundred eggs from the same batch were also monitored for egg hatch (see section 1.2.5 for method).

5.2.3 Monitoring

The experimental pots were searched under a dissecting microscope, every day from day 6 onwards, counting the numbers of each instar present (cadavers were also noted when found). The pots were not searched on days 1 to 5 to avoid damaging or killing eggs and newly hatched larvae. When adult moths began to emerge, they were removed from both the experimental and control pots, and placed individually into 30 ml universal tubes and monitored every day until they died. The dead adults were sexed, and their right mid femur lengths were measured under a dissecting microscope using an eyepiece graticule (see section 4.2.4 for a discussion of why this measure of size was chosen). Thus, a single cohort of individuals was followed through from egg-laying to (unmated) adult death, at four different initial densities.

5.3 Results

5.3.1 Data interpretation

In a small number of instances it was not possible to identify accurately all of the individuals present in a pot, and this led to the following rules being used to condition the data before analysis:

1. **The maximum number of individuals found in a pot was assumed to be the number that had successfully hatched.** To avoid damaging eggs and early 1st instar larvae, the pots were not searched before day 6, when it was certain that all of the viable eggs would have hatched. Therefore it was not possible to know exactly how many eggs had hatched. First instar mortality was very low in all treatments, so it seemed reasonable to assume that the number of individuals in the early samples was equal to the number that had hatched. From previous experiments, the eggs were known to hatch about 4 days after laying, with very little variation in hatching time (see section 6.5.3), therefore all eggs were assumed to have hatched between days 4 and 5.
2. **If the total number of individuals in all stages *increased* between two counts, the number in the first count was adjusted by increasing the number of the smallest instar present in that count.** This adjustment was sometimes necessary in the early stages of the experiment at around the time when larvae were small, and moulting from one instar to the next. Just before moulting, the larvae became very inactive and cryptic, while after moulting they were very active and relatively easy to spot, therefore "missing" individuals were assumed to have been lower instars preparing to moult. This and the previous rule were used to correct 80 counts out of a total of 1546.
3. **Unhatched pupae at the end of the experiment were assumed to have died on the day that the last moth emerged from that pot.** The experiment was ended on day 50 (though the pots were kept for a further 2 weeks, and no more moths emerged from them during that time). It was sometimes hard to tell whether or not a pupa had died, and since it was impossible to track the fates of individual pupae, the point of death could not be determined later. The most reasonable assumption was that the pupa died when the last of the known live pupae emerged. This will unfortunately lead to a slight overestimation of the pupal period in analysis of the data. This rule was applied to 10 pupae.

There were also two replicates which were assumed to be set-up errors, since they contained unexpected initial numbers of individuals. One of these was a pot from the 8 eggs treatment that contained 9 individuals, which was excluded from any analyses. The other was a pot from the 16 eggs treatment that had a maximum of only 8 individuals, and this was reclassified as an 8 eggs treatment for analysis. Thus, analysed sample sizes for each experimental treatment were: 4 eggs – 10 replicates; 8 eggs – 10 replicates (one reclassified from 16 eggs); 12 eggs – 10 replicates; 16 eggs – 9 replicates.

5.3.2 Experimental vs. control treatments

There were no significant differences between experimental and control treatments in time of adult emergence, adult right mid femur length, adult sex ratio, adult life span, or proportion surviving (Table 5.1). Therefore, the remaining analyses are on the combined experimental and control data where appropriate.

	Means				
	Experimental	Control	t	d.f.	p
Day of Emergence	33.2	33.5	-0.687	239	0.493
Adult RMFL (mm)	1.66	1.68	-0.959	239	0.338
Adult sex ratio	0.55	0.54	0.075	69	0.940
Adult life span (days)	5.85	5.79	0.422	239	0.674
Egg to adult survival	0.405	0.558	-1.812	69	0.074

Table 5.1 Tests of differences between experimental and control treatments. RMFL = mid right femur length.

5.3.3 Egg to adult survival

The proportion of eggs that survived to produce adults decreased significantly as initial density increased (Table 5.2). The survival values were proportions and so were arcsine transformed before analysis (Sokal and Rohlf, 1995), the given means are back-transformed values. The egg hatch rate was 0.94.

Treatment	Mean survival	ANOVA
4 Eggs	0.825	F = 30.16
8 Eggs	0.533	d.f. = [3, 67]
12 Eggs	0.262	p < 0.001
16 Eggs	0.177	

Table 5.2 Egg to adult survival for 4 different initial densities.

5.3.4 Time of adult emergence

The time of adult emergence varied significantly between treatments (Table 5.3). In general, development time increased with increasing density, however the mean for 16 eggs is approximately the same as that for the 12 eggs treatment.

Treatment	Mean day of emergence	ANOVA
4 Eggs	31.6	F = 19.74
8 Eggs	32.6	d.f. = [3, 237]
12 Eggs	35.3	p < 0.001
16 Eggs	34.8	

Table 5.3 Time of adult emergence for 4 different initial densities.

5.3.5 Adult Size

Adult size, as measured by right mid femur lengths, differed significantly between sexes and between treatments. A two-way ANOVA showed significant ($p<0.001$) effects of sex and initial density, with no significant interaction. Females were consistently larger than males, and higher density treatments produced smaller adults.

Treatment	Mean Male RMFL (mm)	Mean Female RMFL (mm)
4 Eggs	1.75	1.89
8 Eggs	1.68	1.76
12 Eggs	1.53	1.59
16 Eggs	1.45	1.58

Table 5.4 Mean right mid femur lengths (RMFL) for males and females for 4 different initial egg densities.

5.3.6 Adult sex ratio

There was no significant difference between the numbers of males and females emerging from each pot (paired samples t-test, $t = -0.940$, d.f. = 70, $p = 0.351$), and no significant

effect of treatment on sex ratio (one-way ANOVA, $F = 0.734$, d.f. = [3, 67], $p = 0.535$, using arcsine-transformed sex ratios).

5.3.7 Adult life span

Unmated adult life span decreased with increasing initial egg density (Table 5.5).

Treatment	Mean adult life span	ANOVA
4 Eggs	6.46	$F = 12.02$
8 Eggs	5.85	d.f. = [3, 237]
12 Eggs	5.58	$p < 0.001$
16 Eggs	5.14	

Table 5.5 Adult lifespan for 4 different initial egg densities.

5.3.8 Stage-frequencies

A more detailed analysis of these data is presented in Section 5.4. Figure 5.1 shows the daily mean counts of each life-stage of *P. interpunctella* for each of the four initial densities. As initial density increases, the length of the later larval stages (especially 5th instars) increases — causing greater overlap of the pupal and adult stages — and their survival decreases dramatically. Stage durations and survival up to the 3rd instar remain roughly constant between treatments.

Whenever cadavers were found they were noted, though it was impossible to find cadavers for all of the apparent deaths, since they were often eaten by other larvae. Figure 5.2 shows total counts of cadavers on each day across all replicates, along with mean frequencies of living individuals. In the early stages, most cadavers are found at or slightly after the moult from one instar to the next, while in the high density treatments cadavers are also found throughout the 5th instar and pupal stages. Cannibalism of pupae by late 5th instar larvae was particularly common in the higher density treatments, and many 5th instar larvae were also cannibalised or appeared simply to starve to death over a number of days.

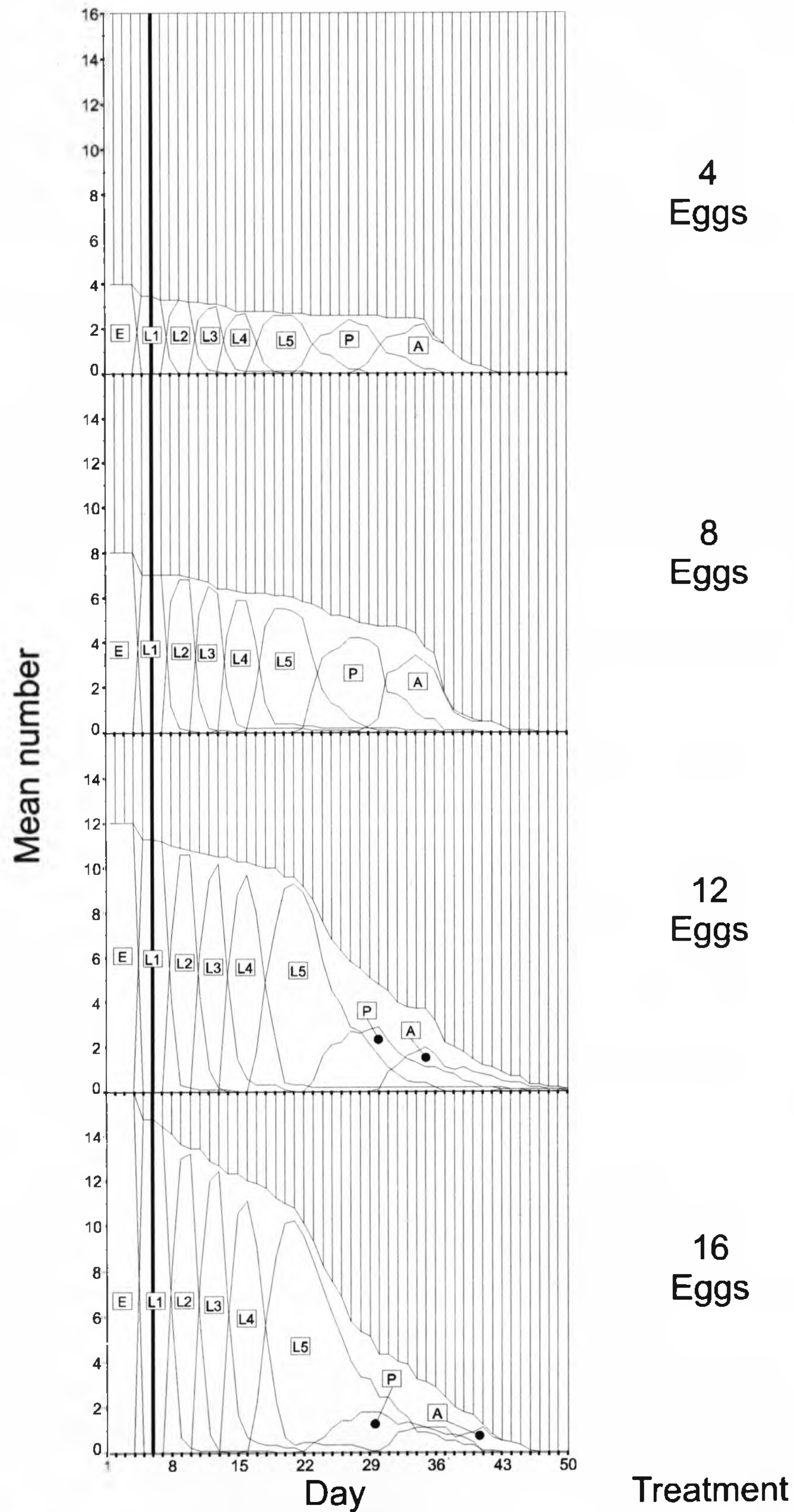


Figure 5.1 Mean daily counts of each stage of a cohort of *P. interpunctella* at four different initial densities. Values before day 6 (heavy line) are estimated (see text). E = Eggs; L1 – L5 = Instars 1 – 5; P = Pupae; A = Adults. The unhatched area represents the total count in all stages.

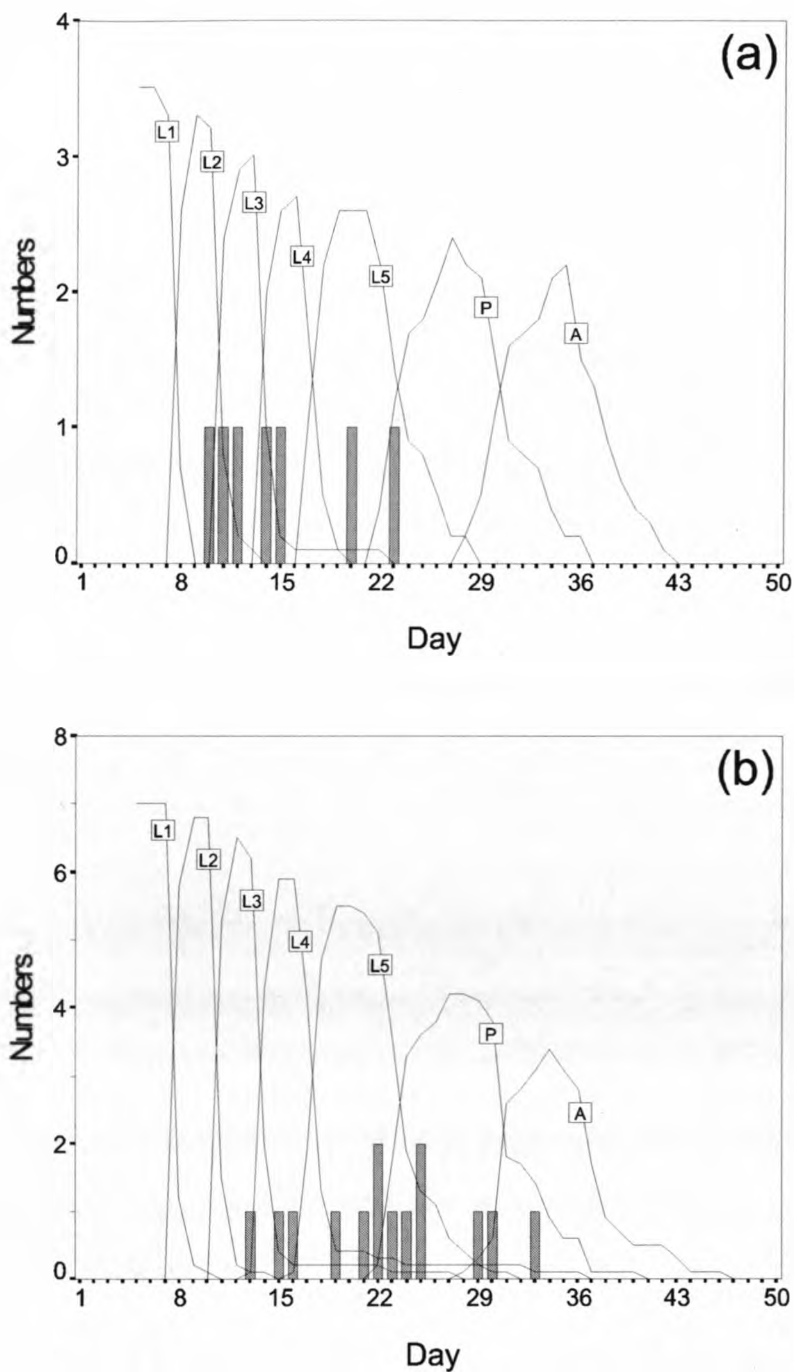
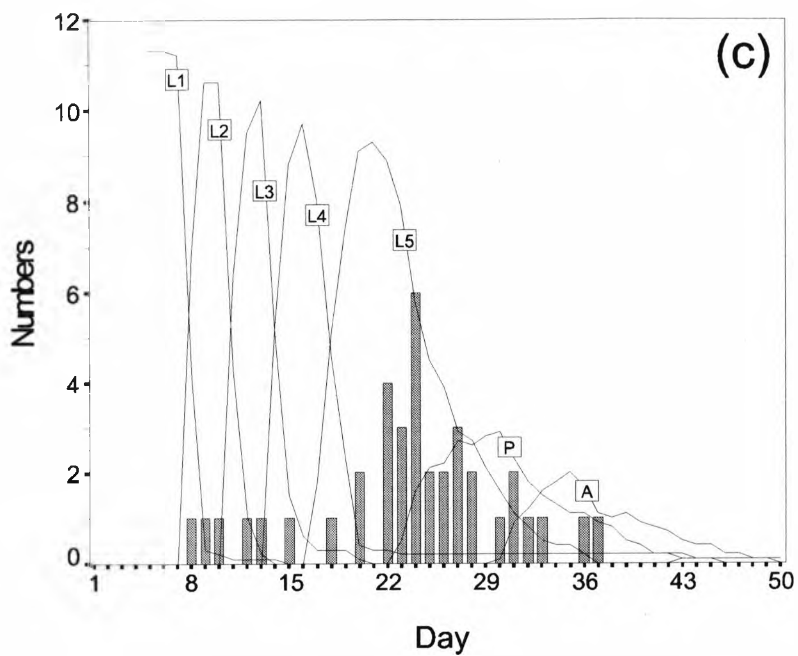


Figure 5.2 Mean frequencies of each stage of *P. interpunctella* from different initial densities with total counts of cadavers. Lines are mean frequencies of each stage, bars are total counts of cadavers from 10 replicates of each treatment. Treatments: **(a)** 4 eggs; **(b)** 8 eggs; **(c)** 12 eggs; **(d)** 16 eggs (9 replicates). Continued on the next page ...



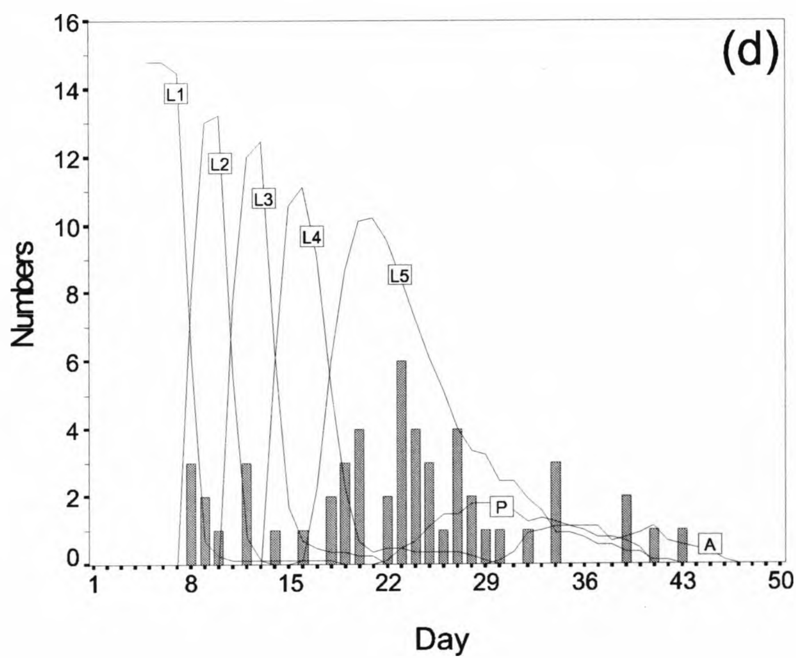


Figure 5.2 continued.

5.4 Stage-frequency analysis

5.4.1 Introduction

Methods for the analysis of stage-based data have developed relatively slowly compared to those available for age-based data. Most early methods were complex, involving the fitting of detailed statistical models to the data using maximum likelihood estimation (Bellows and Birley, 1981; Bellows *et al.*, 1982; Manly and Seyb, 1989; Manly, 1990). However, several recent methods have been developed for analysing stage-frequency data that derive biologically meaningful parameters — such as through-stage survival rates, stage durations etc. — without the need for such complex techniques. In the following analysis, I use two of these recent methods, both by B.F.J. Manly (Manly, 1987; Manly, 1993), to estimate stage durations and through-stage survivals for the experimental data in this chapter.

5.4.2 Terminology

In the two analyses that follow, several parameters are found in both of them, yet different symbols are used for them in the original papers. I have unified the symbols and conventions here for continuity. The parameters common to both methods are:

i = sample number. The first sample is $i = 1$, while $i = n$ for the last sample. In this particular case, since samples were taken every day for the whole course of the experiment, i is also equivalent to time in days.

j = stage number. For the first stage, $j = 1$, whilst for the last stage $j = q$. In both analyses, the first stage was the 1st larval instar.

$f_{i,j}$ = the number in stage j at sample i.

ϕ, ϕ_j = the overall (ϕ), or stage-specific (ϕ_j) survival rate between two samples.

a_j = the mean duration of stage j.

ω_j = the through-stage survival for stage j.

Estimated values are distinguished from observed ones by adding a 'hat' symbol, e.g. $\hat{f}_{i,j}$ = the estimated frequency of stage j at time i.

5.4.3 The Manly (1993) method

5.4.3.1 Description

This relatively simple method, developed by Manly (1993), requires no other information apart from the stage-frequency counts themselves, and makes no assumptions about the distribution of entry times into the first stage. It estimates the rates of transition between one stage and the next, from which other parameters, such as mean stage durations, and through-stage survivals can be calculated. This method does assume, however, that there is a constant daily survival rate during the period covered by the data, and works by finding the value of this daily survival rate that minimises the standard deviation between the observed and estimated stage-frequencies.

A population has q stages and is sampled at times i = 1, 2, 3 ... n. The daily survival rate = ϕ, and the transition rate of stage j-1 to stage j is α_j. The following steps are used to estimate the values of ϕ and α_j:

1. Choose a trial value ϕ' for the survival rate, ϕ.
2. Given that $\hat{\alpha}_q = 0$ (since the final stage can only die), estimate $\hat{\alpha}_{q-1} \ldots \hat{\alpha}_1$ using the formula:

$$\hat{\alpha}_{j-1} = \left\{ \sum_{i=2}^{n} f_{i,j} / \phi' - \sum_{i=2}^{n} f_{i-1,j}(1-\hat{\alpha}_j) \right\} / \sum_{i=2}^{n} f_{i-1,j-1}$$

Equation 5.1

3. Predict values of $\hat{f}_{i,j}$ for j = 2, 3, ... q and i = 2, 3, ... n, using the formula:

$$\hat{f}_{i,j} = \left\{ \hat{f}_{i-1,j-1}\hat{\alpha}_{j-1} + \hat{f}_{i-1,j}(1-\hat{\alpha}_j) \right\} \phi'$$

Equation 5.2

Values of $\hat{f}_{i,j}$ for i =1 and j = 1...q, and for i = 1...n and j = 1 are from the observed values, $f_{i,j}$.

4. Find the standard deviation $s(\phi')$ for the deviations between observed values of $f_{i,j}$ and the predicted values of $\hat{f}_{i,j}$ from step 3.

5. Repeat steps 1–4 using different values of ϕ' to find the value that minimises $s(\phi')$.
6. Use the best value of ϕ' as $\hat{\phi}$ in the following equations to find the stage specific survival rates, $\hat{\omega}_j$, and mean stage durations, $\hat{a}_j$, from the following formulae:

$$\hat{\omega}_j = \hat{\phi}\hat{\alpha}_j / \left\{1 - \hat{\phi}(1 - \hat{\alpha}_j)\right\}$$

Equation 5.3

$$\hat{a}_j = 1 / \left\{1 - \hat{\phi}(1 - \hat{\alpha}_j)\right\}$$

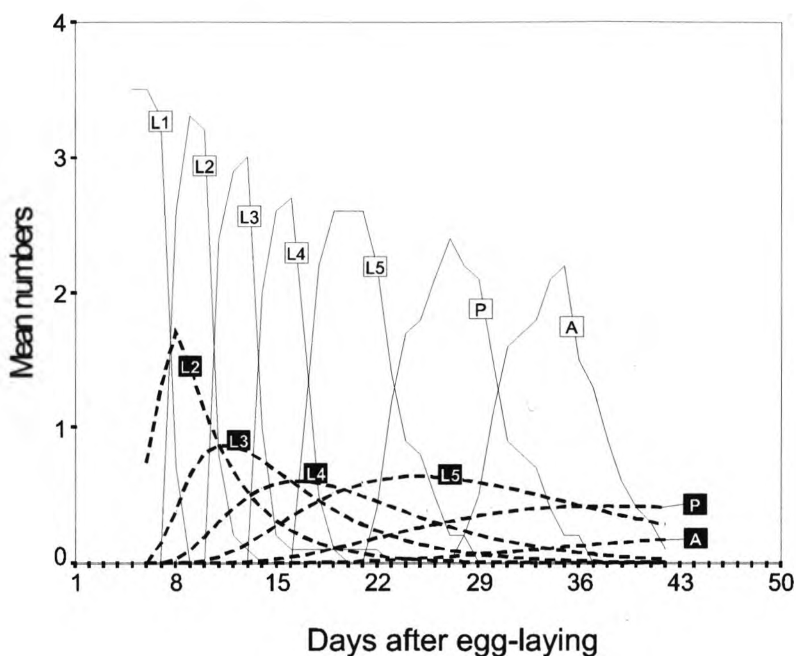
Equation 5.4

5.4.3.2 Analysis

The data were analysed using a custom-written macro for Microsoft Excel 95, following the method outlined above. A listing of the macro is given in Appendix A. The macro performed an analysis on each replicate separately, and then used the parameters from these separate runs to calculate mean parameter values and standard errors for each treatment.

Figure 5.3 to Figure 5.6 show the results of the analyses on the four different initial densities. As the graphs show, the fit of the model seems to be relatively poor, especially in the later stages. Predicted mean adult life span is around 20-30 days, compared with the observed mean of around 6 days (see Table 5.5). However, the analysis does identify trends between treatments which may reflect, if not precisely predict, the underlying real treatment effects. For example, the mean durations of the 4th and 5th instar stages increased with increasing density, while the durations of earlier stages remained roughly constant between treatments. Similarly, the through-stage survival of later instars and pupae decreased with increasing initial density. Both of these trends also appeared in the second analysis, and so probably reflect real trends in the data.

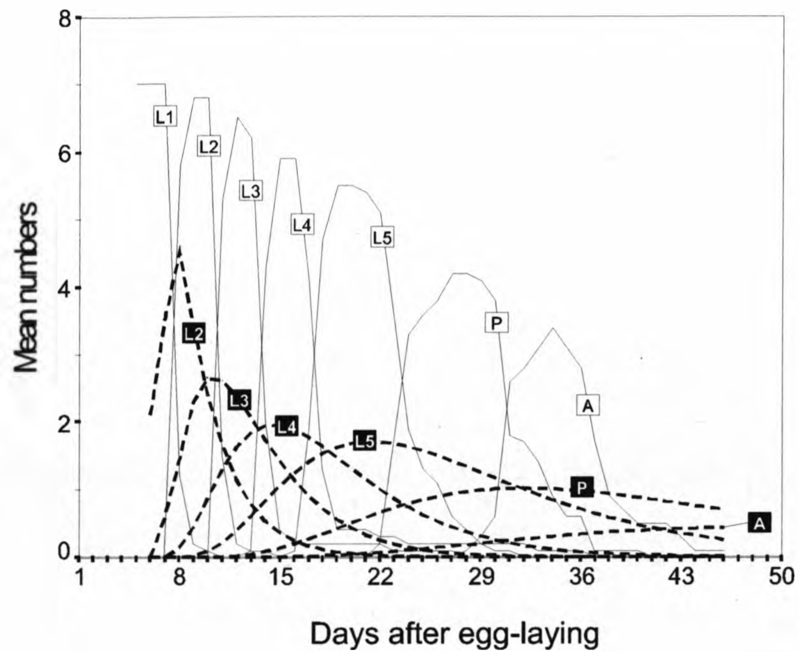
The poor fit of the analysis was probably due to the assumption of a constant daily survival rate for all stages and samples. Figure 5.1 shows that survival clearly varied with time, especially in the later stages, thus the application of a single, constant daily survival rate across the whole sample period was unrealistic in this case. This realisation led to the use of another technique for analysing the data which did allow for different survival rates in each stage, and this analysis is described in section 5.4.4.



Treatment	$\hat{\phi}$	S.E.
4 Eggs	0.9729	0.0018

Stage	$\hat{\alpha}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
L1	0.309	0.027	0.915	0.005	3.316	0.368
L2	0.301	0.020	0.914	0.005	3.268	0.236
L3	0.289	0.020	0.910	0.006	3.414	0.278
L4	0.274	0.015	0.904	0.009	3.478	0.171
L5	0.141	0.020	0.814	0.022	6.633	0.553
Pupa	0.092	0.011	0.749	0.028	9.005	0.654
Adult	0.000	0.000	0.000	0.000	38.978	3.730

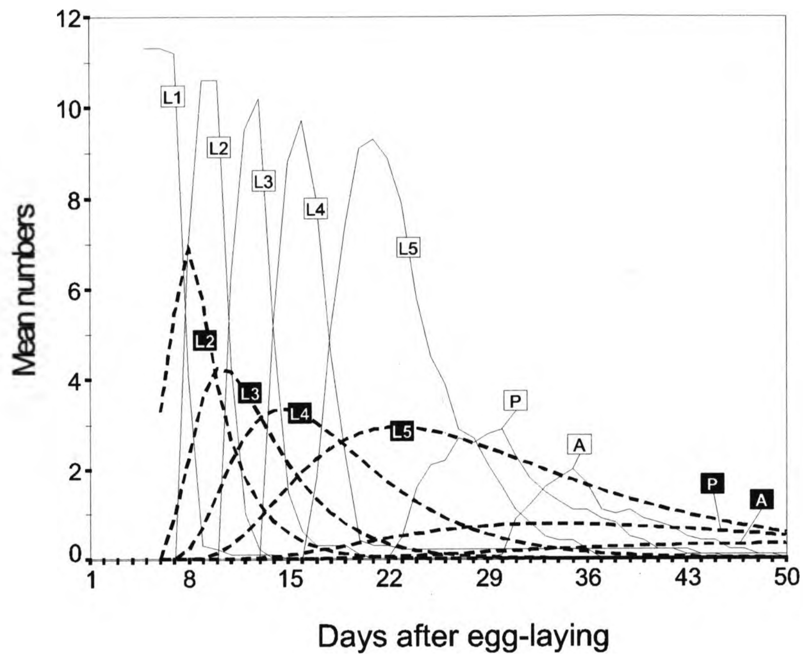
Figure 5.3 The Manly (1993) model fitted to the mean experimental data from the 4 eggs treatment. The graph shows observed frequencies of each instar as fine, solid lines with black text labels, and predicted frequencies as heavy dashed lines with white text labels. The table gives the parameter values derived from the model as means from separate analyses of the 10 replicates of this treatment. $\hat{\phi}$ = estimated mean daily survival rate; $\hat{\alpha}$ = transition rate from one stage to the next; $\hat{\omega}$ = through-stage survival; $\hat{a}$ = mean stage duration.



Treatment	$\hat{\phi}$	S.E.
8 Eggs	0.9600	0.0011

Stage	$\hat{\alpha}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
L1	0.355	0.008	0.895	0.004	2.634	0.051
L2	0.334	0.008	0.888	0.004	2.786	0.057
L3	0.285	0.014	0.869	0.009	3.251	0.164
L4	0.204	0.014	0.826	0.012	4.350	0.244
L5	0.099	0.009	0.693	0.022	7.696	0.488
Pupa	0.057	0.004	0.572	0.014	10.809	0.488
Adult	0.000	0.000	0.000	0.000	25.228	0.808

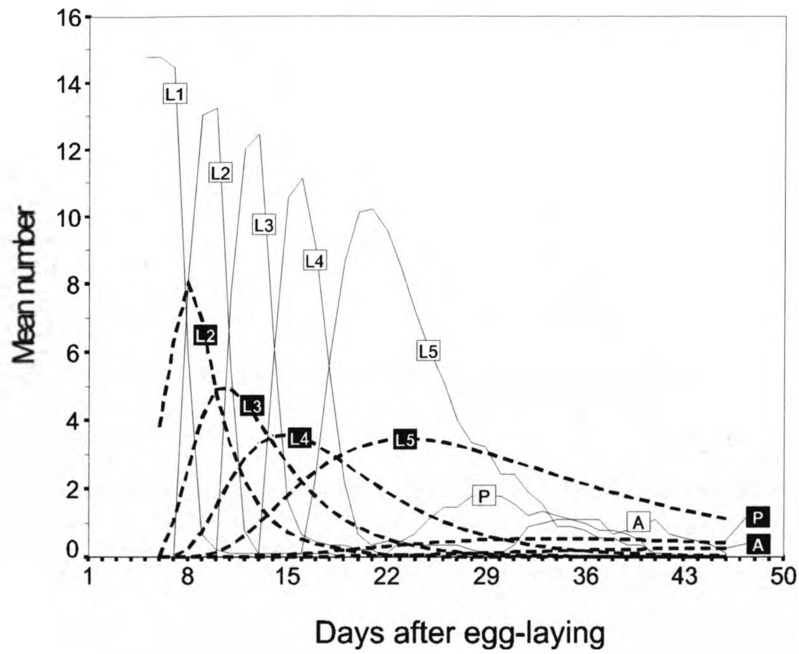
Figure 5.4 The Manly (1993) model fitted to the mean experimental data from the 8 eggs treatment. See Figure 5.3 for description.



Treatment	$\hat{\phi}$	S.E.
12 Eggs	0.9525	0.0008

Stage	$\hat{\alpha}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
L1	0.331	0.011	0.868	0.004	2.776	0.077
L2	0.330	0.006	0.868	0.003	2.771	0.043
L3	0.276	0.008	0.846	0.004	3.237	0.085
L4	0.170	0.013	0.764	0.017	4.963	0.339
L5	0.045	0.007	0.445	0.046	11.625	0.831
Pupa	0.067	0.013	0.529	0.048	10.007	1.057
Adult	0.000	0.000	0.000	0.000	21.116	0.402

Figure 5.5 The Manly (1993) model fitted to the mean experimental data from the 12 eggs treatment. See Figure 5.3 for description.



Treatment	$\hat{\phi}$	S.E.
16 Eggs	0.9492	0.0018

Stage	$\hat{\alpha}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
L1	0.295	0.016	0.845	0.007	3.085	0.166
L2	0.330	0.017	0.858	0.008	2.789	0.116
L3	0.262	0.014	0.826	0.013	3.399	0.173
L4	0.177	0.012	0.763	0.016	4.663	0.240
L5	0.033	0.007	0.342	0.063	12.952	1.107
Pupa	0.072	0.017	0.483	0.094	10.127	1.709
Adult	0.000	0.000	0.000	0.000	19.909	0.754

Figure 5.6 The Manly (1993) model fitted to the mean experimental data from the 16 eggs treatment. See Figure 5.3 for description. There were only 9 replicates of this treatment.

5.4.4 The Manly (1987) multiple regression method

5.4.4.1 Description

This method (Manly, 1987) differs from the previous one in that it allows the stages to have different survival rates. The method of calculating parameters is also different, using multiple regression to estimate the stage-specific survival rates, then calculating other parameters from these estimates. The premise of the analysis is as follows:

A population with q stages is sampled at time $i = 1, 2, \ldots, n$.

$G_{i,j}$ = the number in stage j and higher in the ith sample.

ϕ_j = the survival rate between two sample times for individuals in stage j at the first of these times.

$B_{i,j}$ = the number of individuals in stage j or a higher stage in sample $i+1$ that entered stage j after sample i.

For $i = 2, 3, \ldots, n$ the following relationship should hold:

$$G_{i.1} = B_{i-1.1} + f_{i-1.1}\phi_1 + f_{i-1.2}\phi_2 + \ldots + f_{i-1.q}\phi_q$$

Equation 5.5

In the original paper, the model was designed to analyse multi-cohort data, and so attempted to estimate values of $B_{i-1.1}$ for all samples where new entries to stage 1 occurred. However, for this analysis it is known that all of the larvae are from a single cohort, entering stage 1 between samples i=0 and i=1. Therefore, $B_{i-1.1} = 0$ for $i = 2, 3, \ldots, n$, and Equation 5.5 simplifies to:

$$G_{i.1} = \phi_1 f_{i-1.1} + \phi_2 f_{i-1.2} + \ldots + \phi_q f_{i-1.q}$$

Equation 5.6

Equation 5.6 can be solved using a linear multiple regression method to give estimates, $\hat{\phi}_j$, of each ϕ_j. Now other parameters can be estimated.

The number of entries to stage j between time i and time $i+1$ that are still alive at time $i+1$ is:

$$\hat{B}_{i.j} = G_{i+1.j} - \hat{\phi}_j f_{i.j} - \hat{\phi}_{j+1} f_{i.j+1} - \ldots - \hat{\phi}_q f_{i.q}$$

Equation 5.7

The total number entering stage *j* that survive until a sample time is:

$$\hat{M}_j = \sum_{i=1}^{\gamma.j} \hat{B}_{i.j} + G_{1.j}$$

Equation 5.8

($\gamma.j$ is the last sample to contain new entries to stage *j*)

The mean time of entry to stage *j* is:

$$\hat{\mu}_j = \sum_{i=1}^{\gamma.j} i\hat{B}_{i.j} / \hat{M}_j$$

Equation 5.9

The mean duration of stage *j* is:

$$\hat{a}_j = \hat{\mu}_{j+1} - \hat{\mu}_j$$

Equation 5.10

The stage-specific survival rate for stage *j* is:

$$\hat{\omega}_j = \hat{M}_{j+1} / \hat{M}_j$$

Equation 5.11

5.4.4.2 Analysis

Once again, the analysis was done using a custom-written macro for Microsoft Excel 95. A listing of this macro is given in Appendix B. A potential problem with this method is that regression estimates are not constricted to values between 0 and 1, thus some survival estimates can be greater than 1, which is biologically unrealistic (Manly, 1987). The original paper suggests that if the survival value of a particular stage is estimated as > 1 then the survival for that stage is set to exactly 1, and the analysis is run again without the data for that stage. The macro was written to perform this reanalysis automatically, successively removing stages from the analysis until all remaining survival estimates were equal to or less than 1. Manly also suggested that 'clumping' several successive stages together and reanalysing the data with the total of the individual counts in the clumped classes could also help to reduce the probability of obtaining survival estimates greater

than 1, and the macro also allowed for this refinement. The macro performed the analysis on the data from each separate replicate, then calculated means and standard errors for the estimated parameters.

In Equation 5.8 and Equation 5.9, $\gamma.j$ was used to represent the last sample to contain new entries to stage *j*. When the analysis was performed on these data it was not possible to identify $\gamma.j$ in most cases, since the model was never a completely perfect fit to the observed data. This led to small positive and negative deviations from zero in the estimated values of $\hat{B}_{i,j}$ which would normally have been zero, making it impossible to identify exactly when the last individual entered a stage. Manly (1987) does not mention this problem in his description of the method, and so does not provide a solution. However, I found that the sum of the deviations of $\hat{B}_{i,j}$ for a particular stage tended to be at or close to zero, and this meant that the values produced by Equation 5.8 and Equation 5.9 were not significantly affected if $\gamma.j$ was set to the penultimate sample, n-1.

The analysis was performed twice, once using all of the larval instars as separate stages, and then again with the larvae clumped into two classes: young larvae — instars 1 to 3; and old larvae — instars 4 and 5. The results of these analyses are shown in Table 5.6 and Table 5.7 for the complete and clumped analyses respectively. Clumping the larval stages reduced the number of parameters in the analysis and there were fewer instances where survival estimates were greater than 1 and had to be reset to 1. This, in turn, probably improved the fit of the analysis slightly, at the expense of detailed information about the development of the larvae (Manly, 1987).

4 Eggs

Stage	$\hat{\phi}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
L1	0.978	0.022	0.951	0.049	2.864	0.117
L2	0.986	0.014	0.968	0.032	2.778	0.136
L3	0.960	0.022	0.915	0.040	2.966	0.174
L4	0.939	0.038	0.882	0.070	3.036	0.188
L5	0.991	0.004	0.949	0.023	5.657	0.179
Pupa	0.989	0.008	0.937	0.047	6.621	0.322
Adult	0.904	0.019	—	—	—	—

8 Eggs

Stage	$\hat{\phi}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
L1	1.000	0.000	1.000	0.000	3.002	0.081
L2	0.988	0.007	0.967	0.020	2.892	0.082
L3	0.975	0.013	0.918	0.046	2.944	0.053
L4	0.987	0.008	0.960	0.025	3.866	0.303
L5	0.981	0.008	0.891	0.045	6.352	0.316
Pupa	0.966	0.018	0.844	0.079	7.267	0.330
Adult	0.842	0.018	—	—	—	—

12 Eggs

Stage	$\hat{\phi}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
L1	0.991	0.004	0.969	0.015	3.328	0.073
L2	0.988	0.005	0.967	0.016	3.036	0.076
L3	0.991	0.004	0.971	0.015	3.141	0.120
L4	0.986	0.008	0.932	0.038	3.631	0.513
L5	0.945	0.008	0.552	0.076	10.439	1.526
Pupa	0.913	0.030	0.628	0.097	6.983	0.364
Adult	0.886	0.021	—	—	—	—

16 Eggs

Stage	$\hat{\phi}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
L1	0.980	0.007	0.931	0.025	3.525	0.136
L2	0.982	0.007	0.956	0.016	2.938	0.156
L3	0.976	0.008	0.929	0.022	3.213	0.205
L4	0.986	0.005	0.949	0.017	3.867	0.218
L5	0.928	0.010	0.421	0.078	10.453	1.311
Pupa	0.814	0.049	0.524	0.123	6.166	0.588
Adult	0.687	0.134	—	—	—	—

Table 5.6 Parameter estimates and standard errors for the Manly (1987) multiple regression analysis described in section 5.4.4. The parameter values are means from separate analyses of the 10 replicates of each density treatment (9 replicates of 16 eggs). $\hat{\phi}$ = estimated mean daily survival rate; $\hat{\omega}$ = through-stage survival; $\hat{a}$ = mean stage duration.

4 Eggs

Stage	$\hat{\phi}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
Young Lv.	0.973	0.014	0.846	0.068	8.783	0.361
Old Lv.	0.965	0.019	0.833	0.070	8.478	0.456
Pupa	0.989	0.009	0.935	0.048	6.613	0.323
Adult	0.904	0.019	—	—	—	—

8 Eggs

Stage	$\hat{\phi}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
Young Lv.	0.990	0.003	0.909	0.033	9.452	0.326
Old Lv.	0.985	0.004	0.862	0.038	10.286	0.450
Pupa	0.964	0.018	0.832	0.081	7.228	0.338
Adult	0.843	0.017	—	—	—	—

12 Eggs

Stage	$\hat{\phi}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
Young Lv.	0.992	0.002	0.923	0.014	9.881	0.330
Old Lv.	0.961	0.003	0.516	0.059	15.131	2.134
Pupa	0.906	0.033	0.613	0.098	6.839	0.407
Adult	0.888	0.020	—	—	—	—

16 Eggs

Stage	$\hat{\phi}$	S.E.	$\hat{\omega}$	S.E.	$\hat{a}$	S.E.
Young Lv.	0.980	0.005	0.838	0.036	9.898	0.476
Old Lv.	0.952	0.007	0.437	0.079	15.376	1.240
Pupa	0.790	0.051	0.500	0.125	5.946	0.675
Adult	0.689	0.135	—	—	—	—

Table 5.7 Parameter estimates and standard errors for the Manly (1987) multiple regression analysis described in section 5.4.4. The 5 larval instars have been clumped into 2 classes: young larvae — instars 1 to 3; old larvae — instars 4 and 5. The parameter values are means from separate analyses of the 10 replicates of each density treatment (9 replicates of 16 eggs). $\hat{\phi}$ = estimated mean daily survival rate; $\hat{\omega}$ = through-stage survival; $\hat{a}$ = mean stage duration.

The split between young and old larvae was chosen because, in the full analysis, the through-stage survival and duration of instars 1 to 3 were relatively unaffected by density, while instars 4 to 5 showed marked changes between treatments. Thus the division between young and old larvae was also a division between density-insensitive and density-sensitive stages respectively, for the range of densities tested here.

In both the unclumped and clumped analyses, there was a clear effect of density on both the mean duration and through-stage survival of the later instars, while earlier instars remained roughly constant between treatments. The duration of the last two instars increased from approximately 8.5 days in the 4 eggs treatment to over 15 days in the 12 eggs treatment, though this duration did not increase any further for the 16 eggs treatment, so 15 days appeared to be a maximum limit. Similarly, through-stage survival of the last two instars was almost halved from 0.83 in the lowest density to 0.44 in the highest density. Pupal duration was relatively unaffected between treatments, however, pupal through-stage survival decreased dramatically with increasing density, and this mirrored the experimental observation that many pupae were cannibalised in the high density treatments.

It is hard to relate parameters estimated by this analysis to the observed frequency counts in a way which could provide a good test of the model's fit. There seems to be no way to reconstruct independently the predicted counts in all stages at all samples from a single starting sample. The parameters that would be useful in such a reconstruction, such as the number of entries into each stage at each time, are based on the observed counts from the current sample (see Equation 5.7) and so are not independent of the observed data. Thus the reconstructed data set would always show a close resemblance to the observed data, regardless of whether or not the parameter estimates are close to their real values.

The simplest test is to compare the total mean development time of the immature stages as estimated by the analysis, with the mean emergence time of adults in the experiment and this is done in Table 5.8. The quality of the fit varies between treatments, with the higher density treatments achieving the best fit for both the unclumped and clumped analyses. However, the standard errors of estimated durations encompass those for the

observed means in all treatments, therefore the model estimates of durations do not differ significantly from the observed values.

	Observed		Unclumped		Clumped	
Treatment	Duration	S.E.	Duration	S.E.	Duration	S.E.
4 Eggs	30.56	0.52	27.92	1.12	27.87	1.14
8 Eggs	31.15	0.41	30.32	1.17	30.97	1.11
12 Eggs	33.84	0.75	34.56	2.67	35.85	2.87
16 Eggs	33.90	0.81	34.16	2.61	35.22	2.39

Table 5.8 A comparison of development (egg-to-adult emergence) times in days, for observed data and two different versions of the multiple regression analysis described in section 5.4.4. See text for descriptions of the unclumped and clumped analyses. In both cases, the development duration was calculated as the total of the mean durations of each larval stage + mean pupal duration + 4 days for the egg stage. The observed mean values are calculated from the experimental treatments data only and have had 1 subtracted (since the experiment started on day 1), therefore they differ slightly from the values in Table 5.3.

5.5 Discussion

The experimental data show that density will have significant effects on the sorts of life-history parameters used in stage-specific mathematical models of population dynamics. This is important, not only in the larval stages where density-dependence takes place, but also in the knock-on effects for pupae and adults, in terms of reduced life-span, smaller size, increased mortality etc. (see also chapter 4). In these experiments increased density led to increased development time and mortality in later instars, where density-dependence might be expected to act, but it also led to higher pupal mortality, decreased adult life-span, and smaller adults. The results from the previous chapter show that this will also affect the reproductive potential of the adults, and thus the number entering the next generation. Anderson and Löfqvist (1996) also found that higher larval density increased total development time but that it did not affect adult size (as measured by weight) in *P. interpunctella*. However, see the discussion of the previous chapter for more discussion of Anderson and Löfqvist's paper.

The two stage-frequency analyses presented here demonstrate the problems involved in analysing developmental data. It is not possible to calculate the desired parameters directly from the observed stage-frequency data, therefore all of the available methods

rely on attempting to fit a statistical model. Choosing the correct method to use for a particular data set is largely based on intuitive assumptions about the nature of mortality and development rates in that data set. However, if a model with the wrong assumptions is chosen, then it will produce unrealistic parameter estimates — the difficulty lies in finding a good independent test of the model's fit. Comparisons of different estimation models are rare, but important for finding the best methods (Manly and Seyb, 1989). Here, I compare two different estimation methods in the hope of finding the most appropriate model for the *P. interpunctella* data.

In the first analysis, despite the standard errors of the estimates being very low, it was relatively easy to see, graphically, that the model was a poor fit to the data. In other words, although the analysis produced very consistent estimates, they were consistently *wrong*. It was also relatively easy to spot that the probable reason for the poor fit of the analysis was the assumption of a constant survival rate across all stages, and this led to the use of another method that allowed survival rates of different stages to vary. The second analysis intuitively *appeared* to be a much better fit, but without an independent test of this it is hard to be confident that the parameter estimates truly reflect the real nature of the data. Nonetheless, both analyses showed definite trends in stage durations and through-stage survival rates with increasing density, so it is likely that these are real effects, and not just artefacts of a particular method.

These analyses also confirm that it is probably not necessary to model each larval instar as a separate stage, rather it seems reasonable to divide the larvae into two classes. Young larvae (instars 1 to 3) had a constant development period and low, density-independent mortality, while old larvae (instars 4 and 5) had density-dependent development and mortality rates. The later instars — and especially the final instar — are where the characteristics of the adult moth are determined (Tammaru, 1998). Density-dependence in the later larval stages translates into density-dependence in the adult stage for characters such as body size, fecundity and life-span, and thus the eventual fitness of an individual (see chapter 4).

Cannibalism of pupae by larvae was a significant source of mortality, and occurred in a clearly density-dependent manner. Previous models of *P. interpunctella* population dynamics have considered the pupal stage to be immune to the effects of density (Bjørnstad *et al.*, 1998). However on the basis of these data, future models should include

density-dependent pupal mortality as a factor, and this may significantly affect the dynamics of the system.

The experiments described in this chapter and the last have only dealt with single cohorts of larvae developing from different initial densities at a small scale. While being a useful first step in understanding density-dependence, it probably vastly oversimplifies the fluid and complex nature of responses to density in a large-scale, mixed-age population. Interactions between different developmental stages complicate the definition of density itself — does one count numbers in all stages or in a few dominant stages? Theory has already begun to explore these more complex interactions (Hastings and Costantino, 1987; Hastings and Costantino, 1991; Briggs and Godfray, 1995; Bjørnstad *et al.*, 1998; Briggs *et al.*, *in prep.*), but experimental investigations still have to catch up. More complex experiments using mixtures of different stages will be necessary to understand the full nature of density-dependence in mixed-age populations.

6. Do *Plodia interpunctella* lay "super-eggs"?

6.1 Introduction

This chapter describes a set of experiments designed to test whether *P. interpunctella* adults can manipulate the development rate of the eggs that they lay. This work follows on from the thesis of a previous student at Liverpool, Sarah Lindfield, who described how, under certain conditions, older *P. interpunctella* adults appeared to lay eggs that hatched sooner and developed faster.

6.1.1 Maternal effects

A mother can influence the development of her offspring in many ways, but they all fall under the general term, "maternal effects" (Mousseau and Dingle, 1991; Rossiter, 1991; Bernardo, 1996). A maternal effect is a phenotypic change in offspring caused by genetic or environmental influences acting on the mother (Mousseau and Dingle, 1991). Maternal effects can lead to changes in offspring diapause (Mousseau and Dingle, 1991; McWatters and Saunders, 1996), development rate and other fitness related characters(Fox, 1993a; Fox, 1993b), or can control the expression of polymorphisms in the offspring (Islam *et al.*, 1994).

Maternal effects on the development rate and size of offspring can be due to external influences, such as photoperiod (Giesel, 1988) or food quality (Kerver and Rotman, 1987), or they can be due to individual characteristics, such as maternal size (Fox, 1993b) or age (Fox, 1993a). There are few studies on maternal age effects in insects. Older *Callosobruchus maculatus* females produce eggs that are smaller, less likely to hatch and have lower larval survival (Fox, 1993a). Mousseau and Dingle (1991) mention unpublished data by P.C. Frumhoff which show that older milkweed bugs, *Oncopeltus fasciatus*, produce offspring that develop slightly faster, but the eggs are smaller and have lower survivorship.

6.1.2 Sarah Lindfield's study

In chapter 4 of her thesis, Lindfield (1990) describes a set of experiments studying the effect of maternal age on the development rate of *P. interpunctella* larvae. Since the main

subject of her thesis was the granulosis virus, PiGV, she also compared virus selected lines of *P. interpunctella* against an unselected stock population. Table 6.1 shows the results of her first experiment, in which she collected eggs from groups of 50 moths every day until they had all died, then allowed the eggs to develop for 13 days before weighing and measuring a sample of the resultant larvae. She found that eggs laid from day 4 onwards appeared to develop more quickly, resulting in heavier larvae of a later instar.

		Age of female (days)					
Population		1	2	3	4	5	6
T_1	Wt. (mg)	0.260	0.272	0.363	1.300	1.810	—
	L2 : L3 : L4	9 : 796 : 0	21 : 29 : 0	1 : 49 : 0	0 : 3 : 2	0 : 58 : 1	—
T_2	Wt. (mg)	0.261	0.301	0.360	0.760	2.760	—
	L2 : L3 : L4	5 : 383 : 0	11 : 374 : 0	1 : 210 : 0	1 : 38 : 11	0 : 0 : 17	—
T_3	Wt. (mg)	0.260	0.272	0.281	1.000	2.190	2.510
	L2 : L3 : L4	2 : 48 : 0	111 : 15 : 0	9 : 41 : 0	0 : 1 : 1	0 : 0 : 6	0 : 0 : 39
A_1	Wt. (mg)	0.319	0.451	0.424	0.323	2.829	3.570
	L2 : L3 : L4	0 : 50 : 0	0 : 50 : 0	0 : 50 : 0	0 : 2 : 0	0 : 0 : 12	0 : 0 : 8
A_2	Wt. (mg)	0.519	0.695	0.420	0.368	0.360	3.675
	L2 : L3 : L4	0 : 25 : 0	0 : 50 : 0	0 : 50 : 0	0 : 50 : 0	0 : 3 : 0	0 : 0 : 3
A_3	Wt. (mg)	0.388	0.454	0.371	0.339	3.972	3.851
	L2 : L3 : L4	0 : 50 : 0	0 : 50 : 0	0 : 50 : 0	0 : 3 : 0	0 : 0 : 5	0 : 0 : 4
B_1	Wt. (mg)	0.270	0.441	0.501	0.346	2.867	3.498
	L2 : L3 : L4	0 : 50 : 0	0 : 50 : 0	0 : 50 : 0	0 : 27 : 0	0 : 0 : 9	0 : 0 : 25
B_2	Wt. (mg)	0.343	0.453	0.410	0.421	3.373	3.500
	L2 : L3 : L4	0 : 50 : 0	0 : 50 : 0	0 : 50 : 0	0 : 16 : 0	0 : 0 : 14	0 : 0 : 7
B_3	Wt. (mg)	0.343	0.541	0.513	0.439	3.066	4.450
	L2 : L3 : L4	0 : 50 : 0	0 : 50 : 0	0 : 50 : 0	0 : 4 : 0	0 : 0 : 13	0 : 0 : 4

Table 6.1 Data taken from Lindfield (1990 Tables 4.1-4.3). Mean weights and numbers of each instar in larvae 13 days after laying from different aged mothers. Each replicate population was a group of 25 males and 25 females taken from stock (T), three generations of selection by a virus (A), or two generations of selection by a virus (B).

Lindfield attempted to repeat the experiment using single pairs of moths, but found no age effect on larval development (although low mating success meant that the sample size was low). The third experiment was a repeat of the first, involving groups of moths, but this time she sampled the larvae at different times during their development. She found that as the moths grew older (≥4 days), their offspring were heavier and at a later stage of development, no matter how soon after laying they were examined.

In the work for this chapter I decided to follow up Lindfield's study, to see if I could find out more about these accelerated or "super-eggs" and the conditions under which they were produced. Although my eventual conclusion is that super-eggs probably do not exist and are an artefact of experimental technique, I present the four experiments below in sequence to illustrate the reasoning process that I went through in order to reach that conclusion.

6.2 Experiment 1: pilot experiment

6.2.1 Introduction

This was designed as a quick test to see if I could reproduce the effects found by Lindfield (1990). I used many different treatments with small sample sizes in order to narrow down the options for a more in-depth experiment. In particular, I wanted to see whether adult group-size was important, and also whether continued access to a male had any effect.

6.2.2 Method

For this experiment I wanted to collect eggs from single or small numbers of moths, so I designed a scaled-down version of the "egg-machine" used for maintaining the stock cultures described in chapter 1. This mini egg-machine is illustrated in Figure 6.1. Moths were taken from the 25°C stock cultures at late fifth instar stage, sexed and put into individual 30ml universal tubes, with a small amount of food. The tubes were checked every day to see whether adults had emerged in them. Newly emerged adults were placed into the mini egg-machines in a variety of densities and sex ratios: in half of the treatments there were equal numbers of males and females at densities of 1,5, and 10 individuals of each sex. In the other treatments one female was put into a universal tube with a male, and they were watched until they mated (pairs that did not mate within the first hour were discarded). When the pair separated, the mated female was placed in a mini egg-machine either on its own, or with 9 or 19 unmated females. By doing this I hoped to see the effect of group size on individual females, and to see whether continued access to mates was a factor. Unfortunately, none of the females mated in this way went on to produce any eggs.

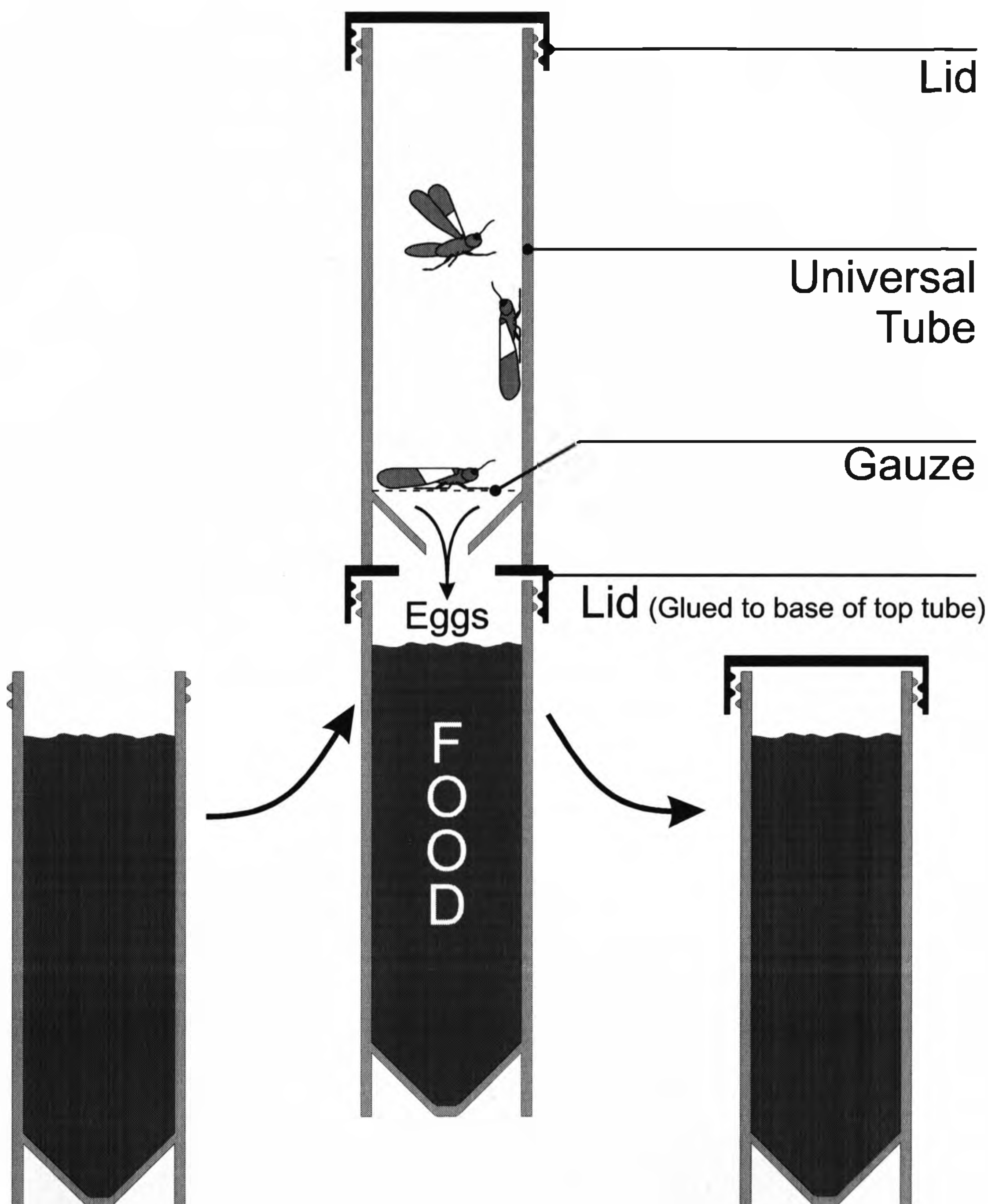


Figure 6.1 The mini egg-machine used for the pilot experiment described in section 6.2. Moths were placed in the top Universal tube, which had a hole in the bottom and another lid (also with a hole) glued to the base. This allowed fresh, food-filled tubes to be screwed onto the bottom of the apparatus to collect the eggs each day. A disk of wide-meshed gauze allowed the eggs to drop into the tubes below while preventing dead moths from blocking the hole.

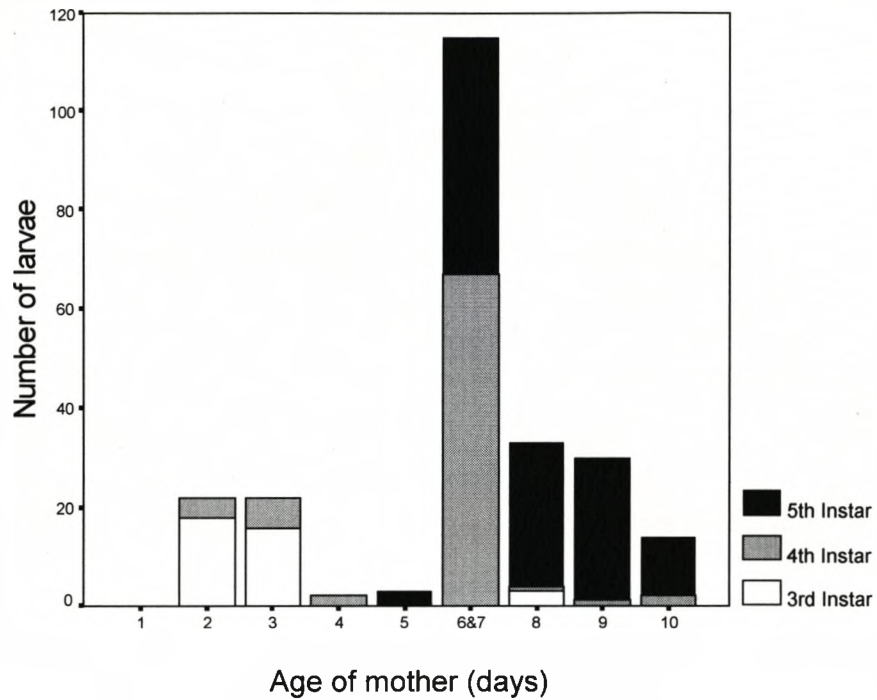
The tubes under each mini egg-machine were changed every day until the last moth had died. The tubes from the mini egg-machines were then left for either 14 or 18 days, then their contents were emptied onto a hotplate at a low setting and the numbers of each instar driven out were counted. The whole of this experiment (as with all of those in this chapter) was conducted in a constant temperature room held at 25 ± 1 C. Humidity was not controlled but remained at around 65 % R.H.

6.2.3 Results

Many of the different treatments that were set up failed to produce any eggs. However this was partly expected, since this was a pilot experiment designed to establish the correct techniques and conditions for a larger experiment. Of the replicates left to develop for 14 days after laying, two replicates of the treatment containing one male and one female laid eggs, and these results are illustrated in Figure 6.2. It was not possible to collect eggs on day 6, so the eggs from days 6 and 7 are combined (the larvae from these days were collected 14 days after day 6). In both cases, the predominant developmental stage 14 days after laying was 3rd instar from young mothers (<5 days old) and 4th and then 5th instar as the mothers got older (>5 days old). Egg laying also appeared to occur in two peaks at days 2-3 and days 6-9 (although some of this was due to days 6 and 7 being combined).

The treatment containing 10 males and 10 females also produced eggs, but the stress caused by putting so many moths into such a small tube meant that they had all died by day 5, before any age effect could become apparent. Some of the replicates left to develop for 18 days had also produced eggs, however almost all of the larvae had reached the final, 5th instar by the time the tubes were sampled. It was therefore impossible to see any maternal age effect on development time in these replicates.

(a)



(b)

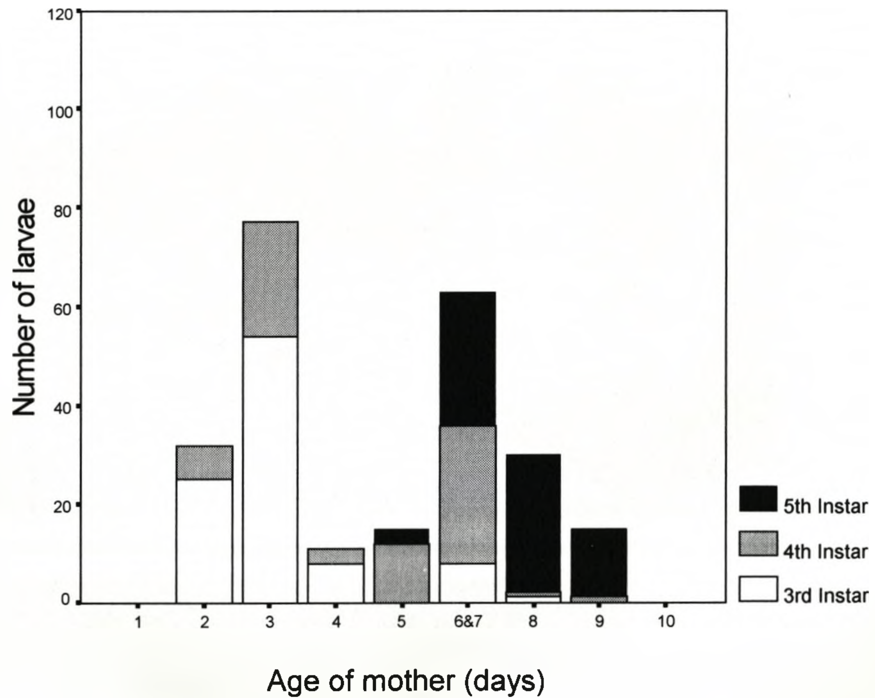


Figure 6.2 The developmental stages of larvae 14 days after laying, against age of mother. The two graphs (a and b) represent the offspring of two different pairs of moths. The bars for each instar are stacked so that the total bar height represents the total number of larvae collected each day.

6.2.4 Discussion

The results of this pilot experiment appeared encouraging, despite the rather small sample sizes. The abrupt change in the developmental stage of larvae as the mother became older seemed to confirm that she was somehow manipulating the development rate of her offspring. What was perhaps surprising was that this effect was found from single pairs of moths, whereas Lindfield (1990) had only found an age effect when the parents were in large groups. She found that the change in larvae occurred at day 4, while these results suggest that the threshold lies at around days 5-6, however this difference was probably due to running the experiments at different temperatures (25 C for this experiment and 28 C for Lindfield's).

The other notable feature of these results was the long egg-laying period. The moths continued to lay eggs until day 9 or 10, whereas Lindfield's moths rarely laid eggs after day 5. The reason for this difference is not quite clear, although some of it could be due to differences in temperature and density.

6.3 Experiment 2: the effect of male presence on super-egg production

6.3.1 Introduction

The pilot experiment had shown that maternal age might have effect on larval development rate. Moreover, it had shown that this effect could occur with single pairs of moths, in contrast to Sarah Lindfield's results. The next step was a more rigorous experiment designed to verify the pilot experiment and to try to identify the causes of super-egg production. The super-eggs were produced towards the end of the female's life (after day 5), so one possibility was that females were running out of sperm and laying parthenogenetic eggs which might have a different development pattern to normal eggs (parthenogenesis occurs in many lepidopteran species (Cockayne, 1938; Suomalainen, 1962)). If this were the case, then females which only had access to a mate at the beginning of their lives should have been more likely to run out of sperm and so lay super-eggs than females that had constant access to a mate. This was the premise for the experiment described here.

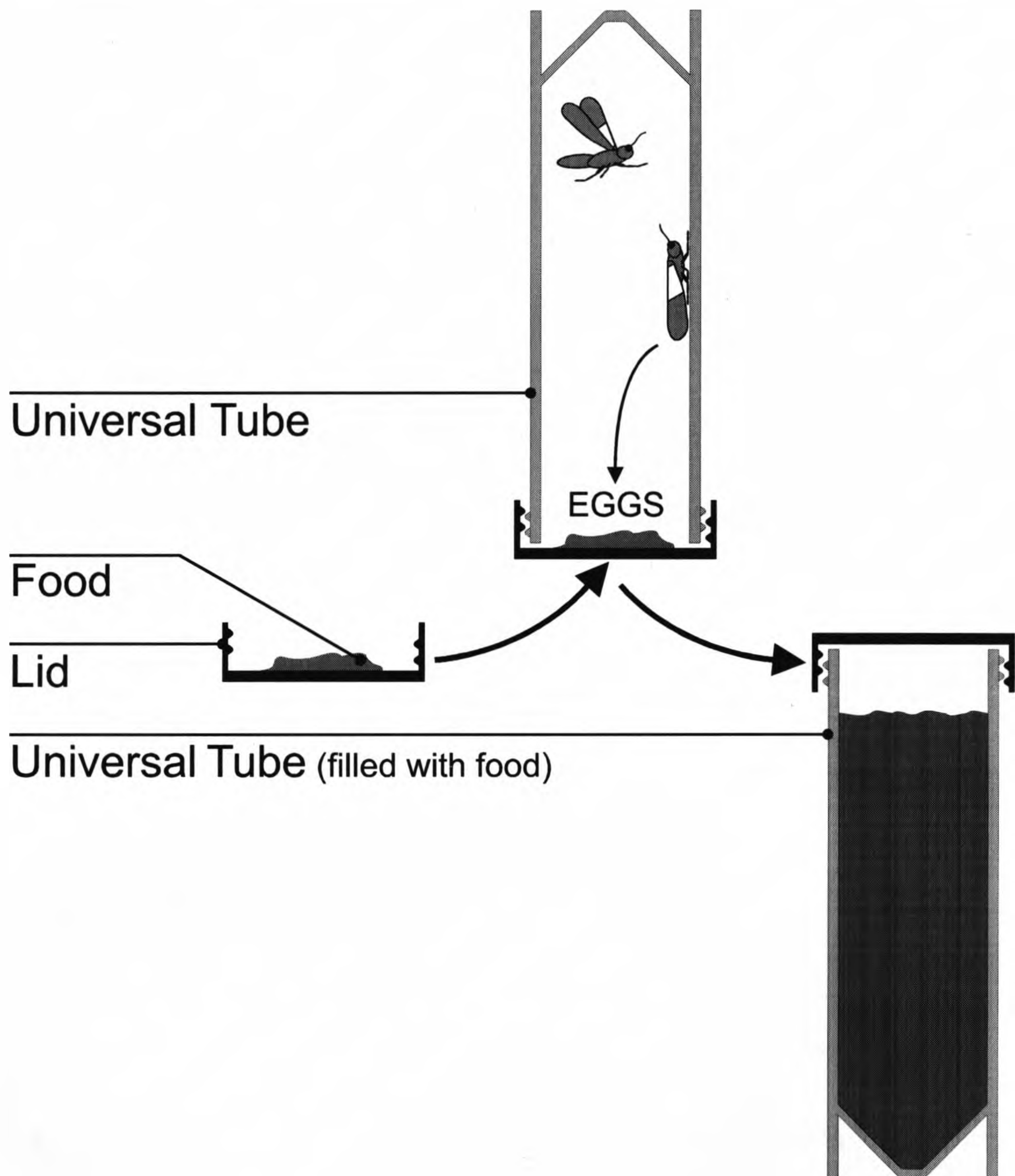


Figure 6.3 The method of collecting eggs for the experiment described in section 6.3. Pairs of moths were placed into universal tubes standing on their lids, with a small amount of food in the lids for the moths to lay into. The moths tended to stay towards the top of the tube, so it was possible to change the lid without disturbing them, by gently unscrewing the lid and replacing it with a new one. The old lid was then screwed onto the top of a food-filled tube, so that the eggs could develop.

6.3.2 Method

Moths were taken from stock culture at late fifth instar stage, sexed and put into individual 30ml universal tubes, with a small amount of food. The tubes were checked every day to see whether adults had emerged in them. It was felt that the mini-egg machines used in the pilot experiment were needlessly complicated, so a simpler method of collecting eggs was devised, illustrated in Figure 6.3. Pairs of moths were set up in tubes and left to mate for 24 hours, then the males were removed from half of the tubes. Eggs were not collected from this first day, since the two treatments had not been set up yet — *P. interpunctella* only start to lay eggs about a day after mating, so very few eggs would have been laid during the first day anyway (see Figure 6.2 and Lindfield (1990)). As before, the eggs were collected every day of the adults' lives and were left to hatch and develop in food for two weeks before collecting the larvae over a hotplate. As well as counting the numbers of each instar, the larvae were weighed collectively on a top pan balance.

6.3.3 Results

There were no significant differences between the two treatments ("single female" and "pair") for any of the following attributes: total number of larvae produced ($t = -0.876$, $p = 0.280$); total numbers of 3rd ($t = -0.909$, $p = 0.199$); 4th ($t = -0.117$, $p = 0.554$); or 5th ($t = 0.288$, $p = 0.418$) instars (degrees of freedom = 39 for all of these independent samples t-tests). Since there was no difference between the treatment groups, the following graphs were based on the combined data from both treatments.

Figure 6.4 shows the mean distribution of instars 14 days after laying, against the age of the mother. Comparing this graph with Figure 6.2 from the pilot experiment shows a similar pattern in the appearance of 5th instar larvae from older mothers. The numbers of 5th instars are very much lower than in the pilot experiment, however their distribution is significantly different from a random, Poisson distribution (Kolmogorov-Smirnov test, $p < 0.001$) that might be expected if they were the result of accidental external contamination of the experiment (Sokal and Rohlf, 1995). There is also a difference in the pattern of egg-laying, with a continuous decline in the number of eggs with age, rather than the two peaks found in the pilot experiment.

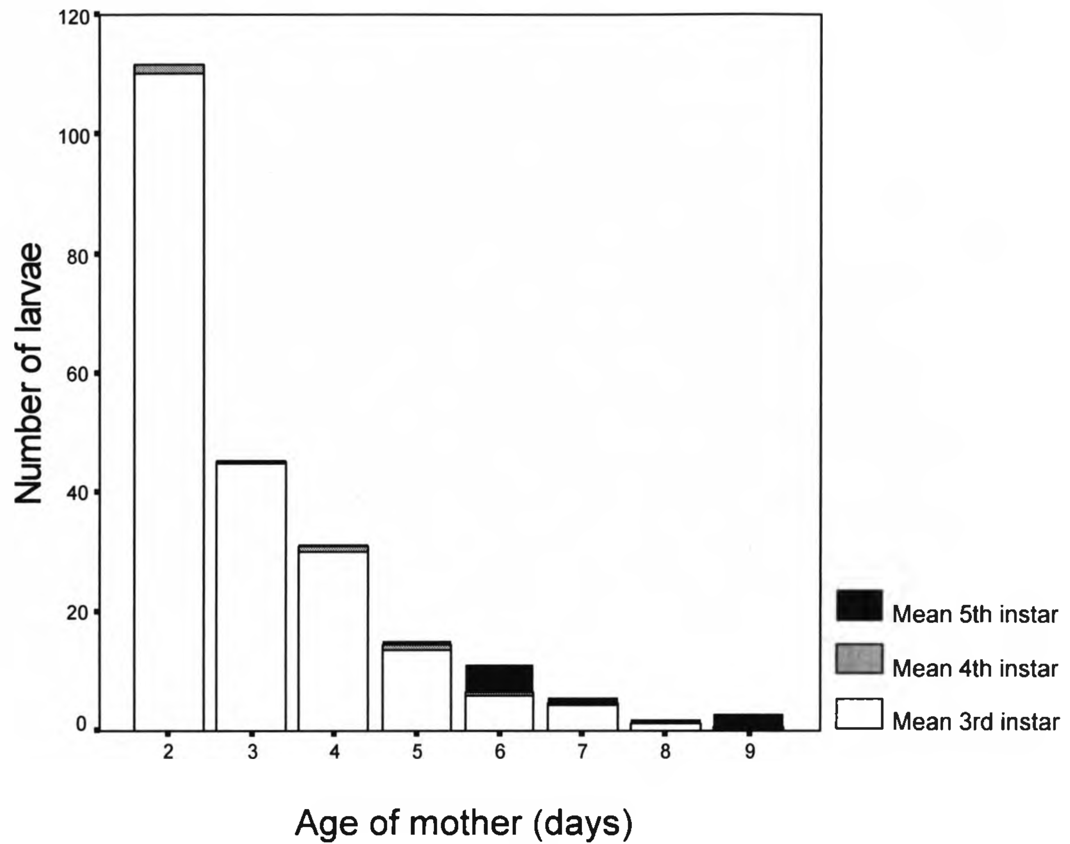


Figure 6.4 The mean numbers of different instars from eggs laid by different aged mothers, 14 days after laying. The bars for each instar are stacked so that the total bar height represents the mean total number of larvae collected each day.

The changes in the characteristics of the offspring with the age of the mother are clearer in the next two graphs (Figure 6.5 and Figure 6.6). Both the proportion of 5th instars (Figure 6.5) and the mean weight of larvae (Figure 6.6) increase significantly from day 5 onwards.

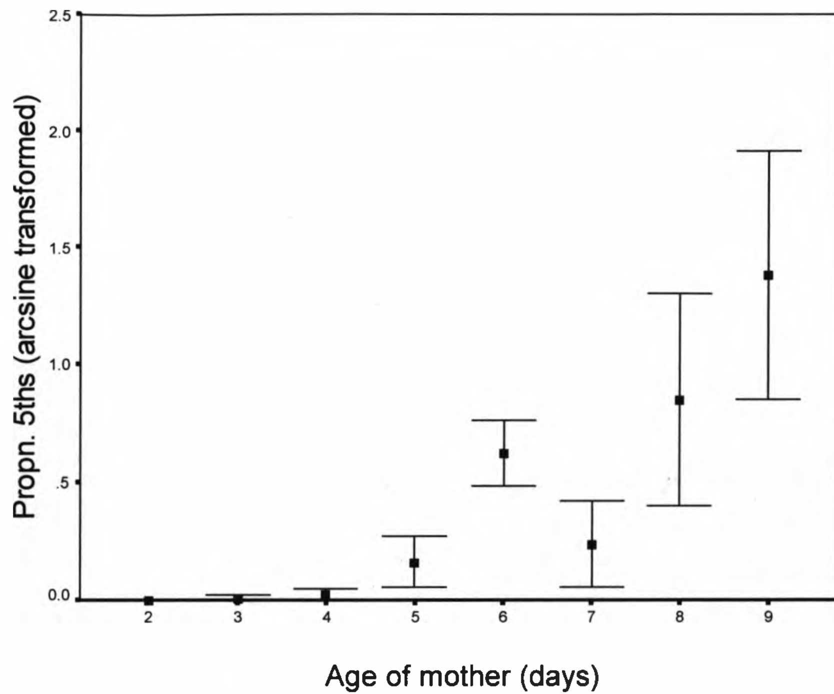


Figure 6.5 The mean arcsine transformed proportion of 5th instar larvae developing from eggs laid by mothers of different ages, 14 days after laying, in experiment 1. Error bars are 95% confidence limits.

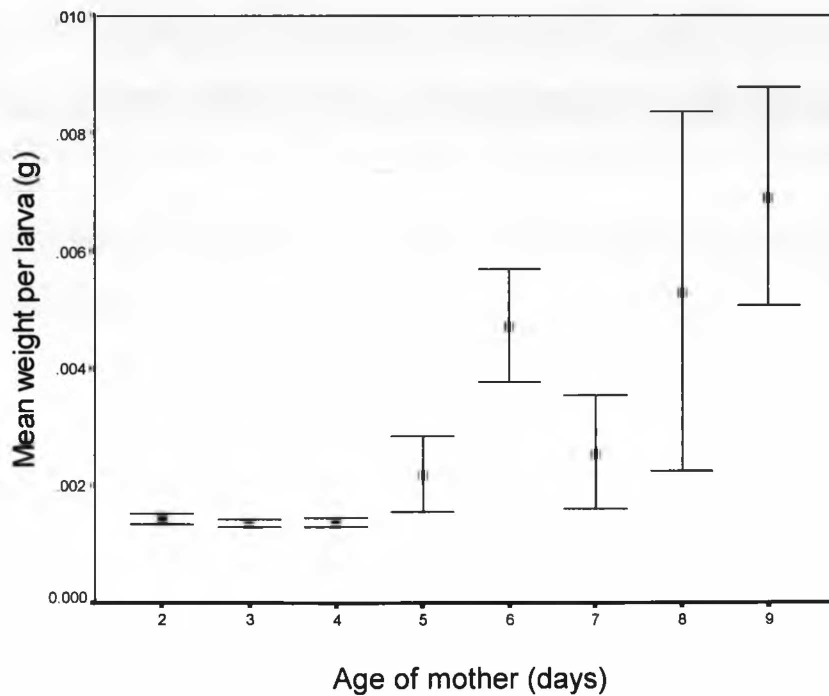


Figure 6.6 The mean weight of larvae developing from eggs laid by mothers of different ages, 14 days after laying, in experiment 1. Error bars are 95% confidence limits.

6.3.4 Discussion

There is a definite change in the characteristics of larvae produced by the moths as they grew older. Larvae developing from eggs laid after day 5 were heavier and at a later instar, than those from earlier days eggs. These later larvae are probably the same type as those found by Lindfield, and so represent some form of super-egg that can develop faster than normal eggs. There was no difference between the treatments, so continued access to mates did not appear to affect a female's ability to produce these super-eggs.

The numbers of super-eggs produced in this experiment were much lower than in the pilot experiment, though still significant. This could be due to the low sample size in the pilot experiment — the two successful replicates may by chance have been exceptional cases. However, there is also a difference in the pattern of egg-laying between the two experiments, which suggests that some change in the experimental methods has led to a change in the moths' behaviour. In this experiment, the majority of eggs were laid before day 5, when the age effect became apparent, while in the pilot experiment at least half of the eggs were laid after this threshold. In the pilot experiment, the moths were not in direct contact with a suitable substrate for their eggs to develop in, while in this experiment they were able to lay directly into the food. This difference may have caused the moths in the pilot experiment to lay fewer eggs in early days, in the expectation that they might find a more suitable laying site later. As they neared the end of their lives without finding a suitable substrate, the moths' best strategy would have been to lay their remaining eggs anyway in the hope that some would survive, and since the moths were older at this stage, some change in their physiology might make them lay super-eggs.

An alternative explanation might be that super-eggs are an adaptation to cope with adverse laying conditions. Faster developing eggs would hatch sooner and might be able to reach a suitable substrate before predation, or other environmental factors killed them. The fact that super-eggs are only produced by older moths may indicate that they take longer to develop inside the female than normal eggs, and so have to be laid later.

6.4 Experiment 3: the effect of food availability on super-egg production

6.4.1 Introduction

The proportion of super-eggs found in experiment 2 was very much lower than in the pilot experiment (though still significant). One possible reason for this difference was that, in the pilot experiment the moths were not in direct contact with a suitable substrate for the eggs, i.e. food. The production of super-eggs might somehow be a reaction to unsuitable conditions for laying normal eggs. Experiment 3 was designed to test this hypothesis.

6.4.2 Method

The method for this experiment was broadly similar to experiment 2. Mating pairs of moths were set up in tubes, as before, but this time the males were not removed. There were two treatments: in the "no food" treatment, the lids of the tubes were carefully cleaned, and contained no food, while the in the "with food" treatment, the lids contained a small amount of food, as in the previous experiment. Three days into the experiment I noticed that the moths (and especially those in the "no food" treatment) were laying eggs onto the sides of the universal tubes as well as into the lids. I realised that this could provide a possible source of cross-contamination between days, since eggs from previous days' egg-laying could fall into, or hatch and crawl into later days' egg batches. Therefore, I began changing both the lids *and* the tubes for clean ones every day starting from day 3 (see Figure 6.7).

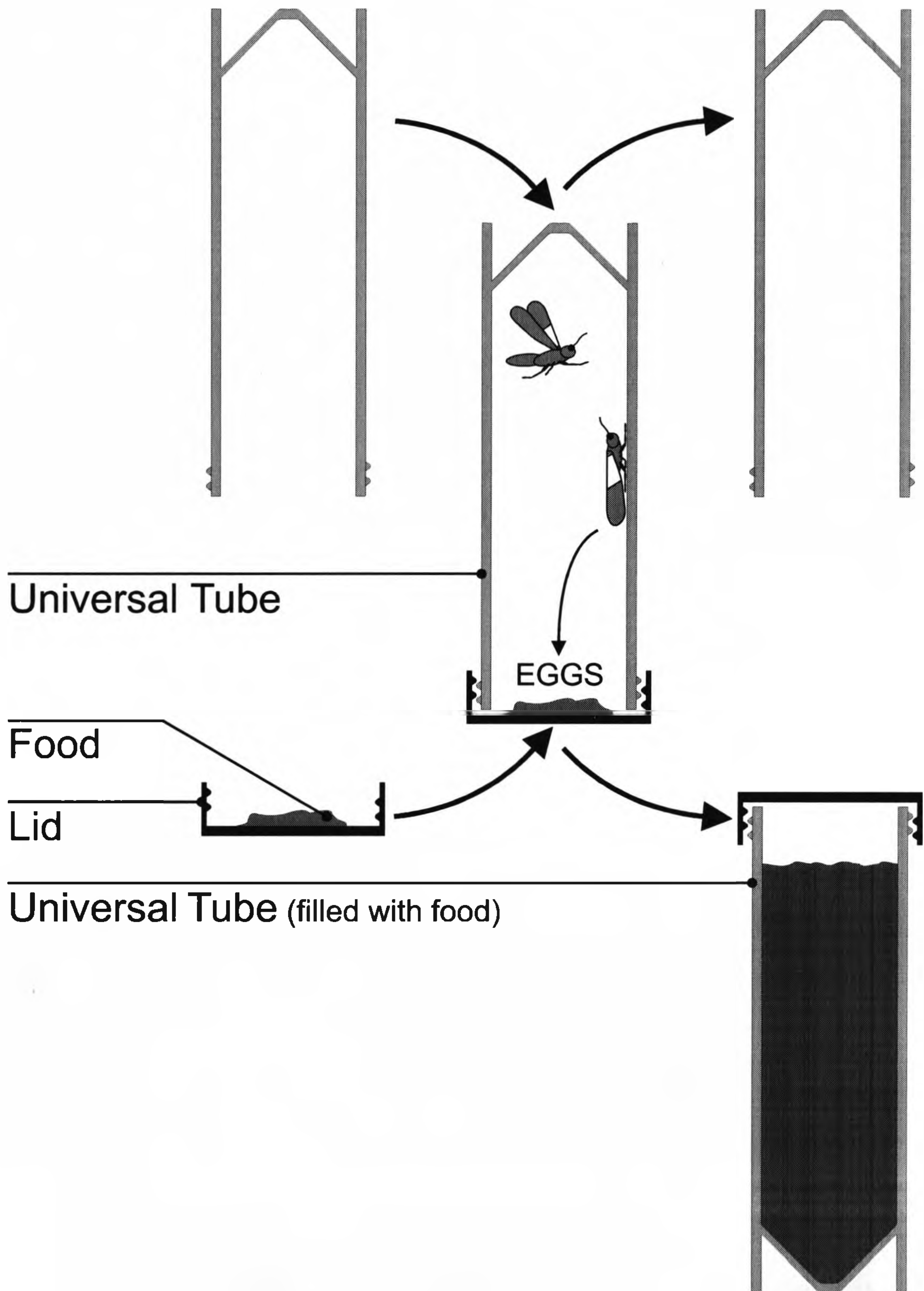


Figure 6.7 The revised protocol used in experiment 3 from day 3 onwards. It was basically the same method as that illustrated in Figure 6.3, except that the moths were transferred to a new universal tube every day, and the old tube was discarded.

6.4.3 Results

Table 6.2 shows that there was a significant difference between treatments in the total number of larvae produced, however, this difference was entirely due to a difference in the numbers of 3rd instars. Figure 6.8 shows that the two treatments differed significantly only on day 2 and that the difference was possibly due to a different pattern of egg-laying between the two treatments (the moths in the "no food" treatment appeared to lay smaller numbers of eggs over a longer time period). This difference became non-significant in a repeated measures MANOVA in which age of mother and treatment were factors, nonetheless the rest of the graphs in this section consider each treatment separately.

	No Food		With Food		T-Test		
	n	*mean*	*n*	*mean*	*t*	*d.f.*	*p*
No. 2nd Instar	21	2.33	27	3.52	-1.051	46	0.299
No. 3rd Instar	**21**	**172.62**	**27**	**239.70**	**-2.923**	**46**	**0.005**
No. 4th Instar	21	15.14	27	16.81	-0.310	46	0.758
No. 5th Instar	21	0.76	27	0.30	2.017	30.05†	0.053
Total Larvae	**21**	**190.85**	**27**	**260.33**	**-2.882**	**46**	**0.006**

Table 6.2 Independent samples t-tests between treatments in experiment 3 for the mean lifetime numbers of different instar larvae 14 days after laying. Bold rows indicate a significant difference between the treatments ($p < 0.05$). †The degrees of freedom were reduced due to unequal sample variances.

The distribution of instars from different age mothers is shown in Figure 6.9. Notice that there are now hardly any 5th instars, and that the offspring from any age mother now consist almost entirely of 3rd instars, even in the "with food" treatment that gave 5th instars in experiment 2 (compare Figure 6.9b with Figure 6.4). The distribution of 5th instars is now not significantly different from a Poisson distribution (Kolmogorov-Smirnov test, $p = 1.00$), and so is probably due to random contamination. Also included for comparison with experiment 2 are graphs of the mean proportion of 5th instars (Figure 6.10) and the mean larval weight (Figure 6.11), and these confirm that there is no change in the characteristics of the larvae as the mother ages.

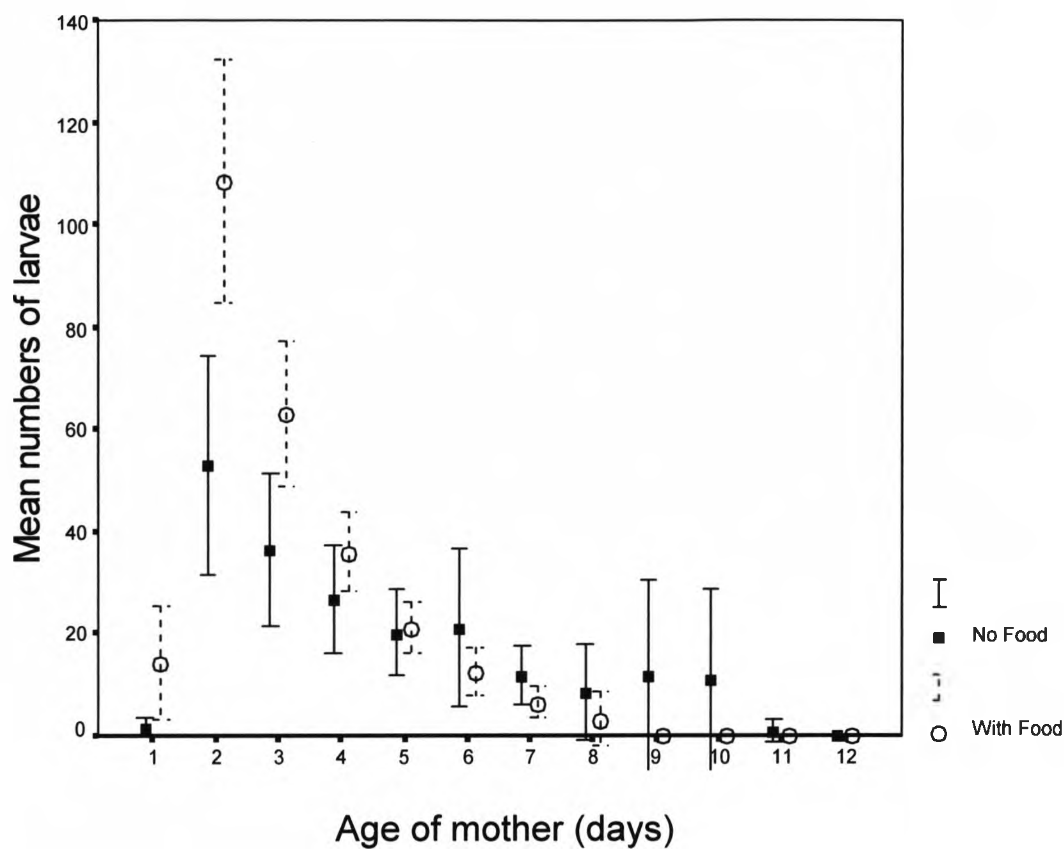
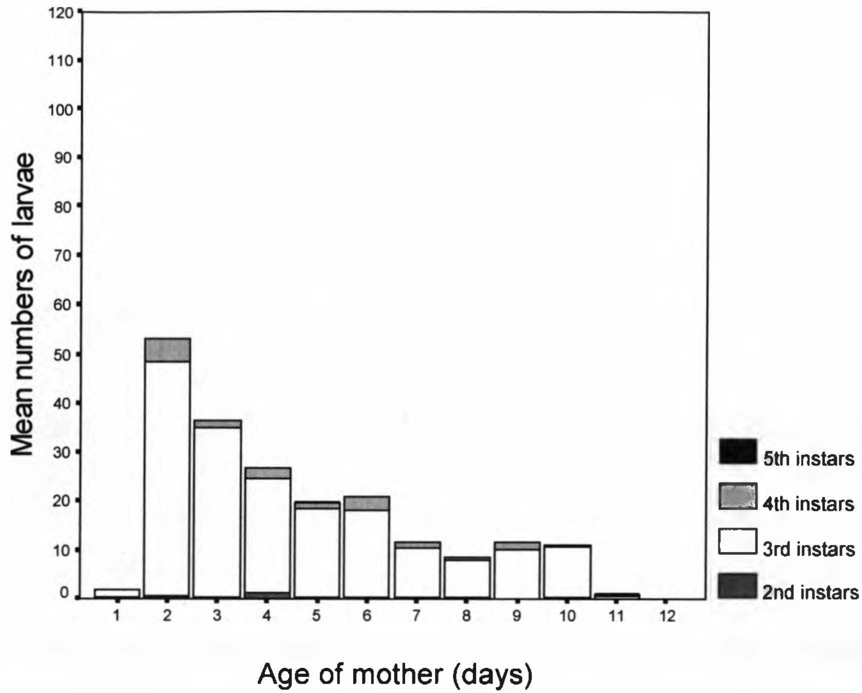


Figure 6.8 The mean numbers of larvae (all instars combined) 14 days after laying by mothers of different ages, in experiment 3. Error bars are 95% confidence limits.

(a)



(b)

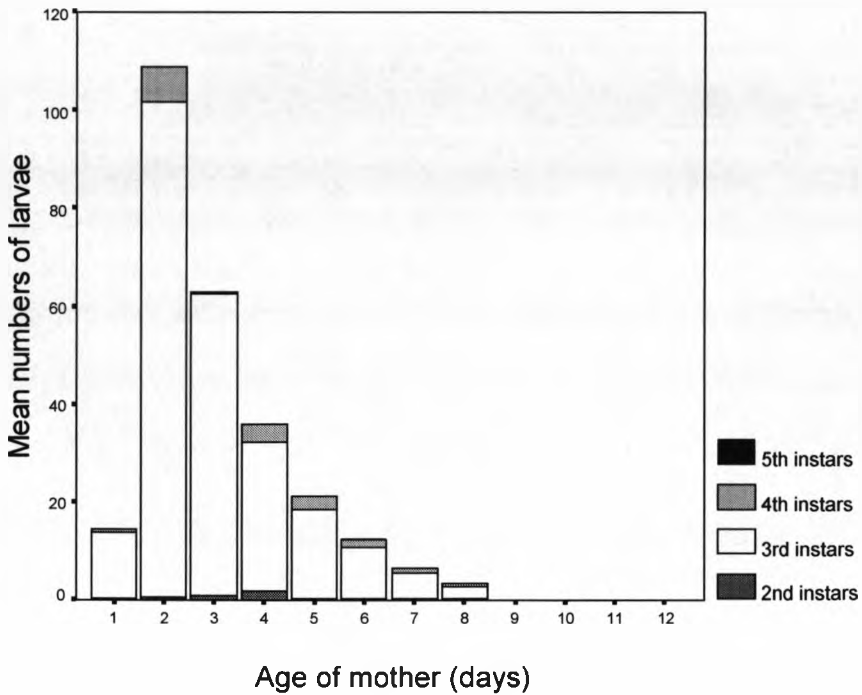


Figure 6.9 The mean numbers of each instar 14 days after laying by different aged mothers in experiment 3. (a) The "no food" treatment. (b) The "with food" treatment. The bars for each instar are stacked so that the total bar height represents the mean total number of larvae collected each day.

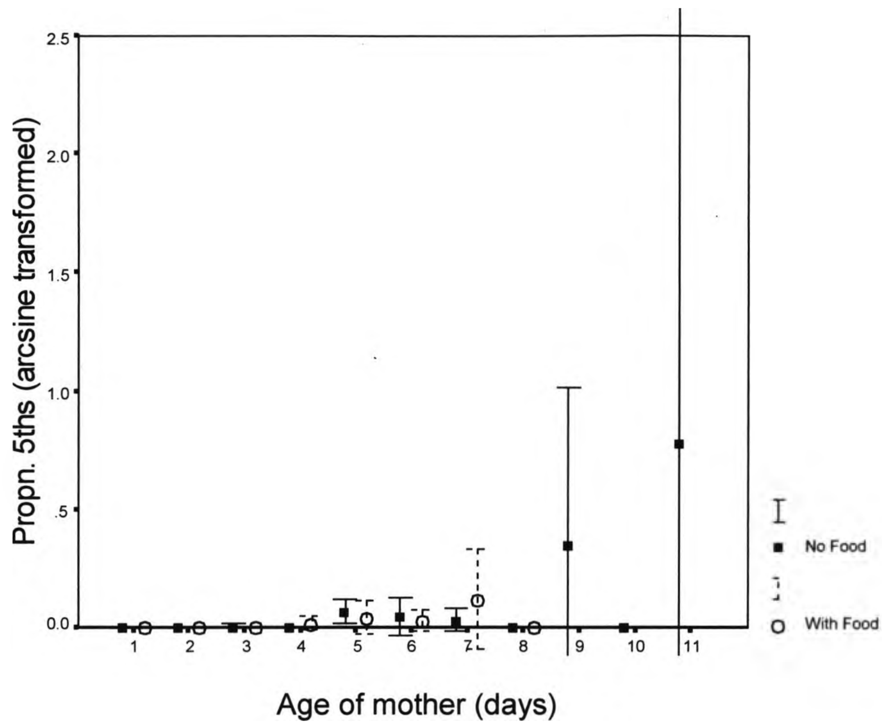


Figure 6.10 The mean arcsine transformed proportion of 5th instar larvae developing from eggs laid by mothers of different ages, 14 days after laying, in experiment 3. Error bars are 95% confidence limits.

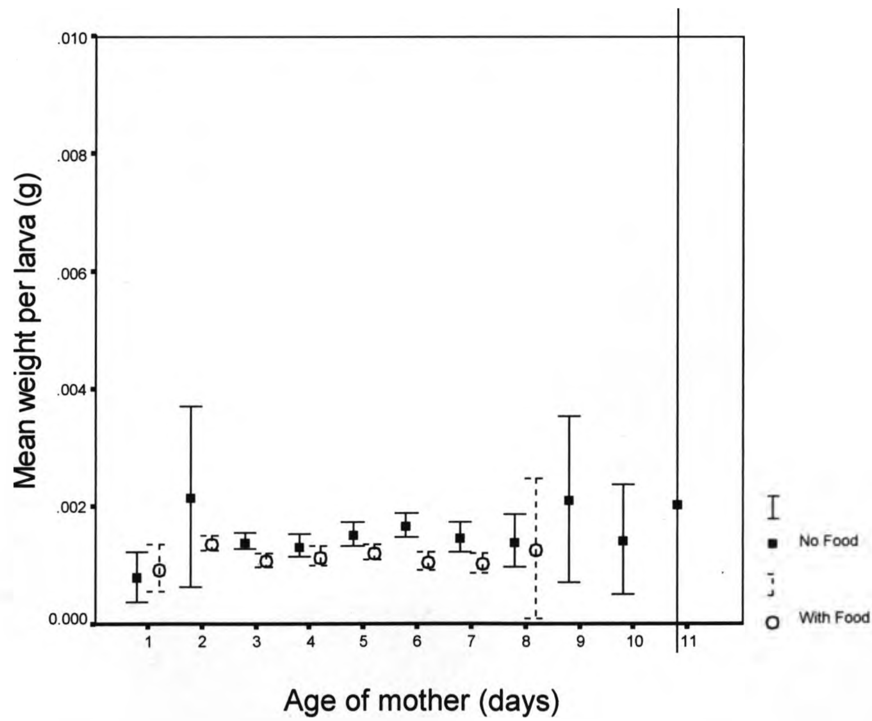


Figure 6.11 The mean weight of larvae developing from eggs laid by mothers of different ages, 14 days after laying, in experiment 3. Error bars are 95% confidence limits.

6.4.4 Discussion

The disappearance of super-eggs from this experiment compared to experiment 2 is telling. Even the "with food" treatment — which is essentially identical to the "pair" treatment in experiment 2 — showed no evidence of super-egg production. The only difference between the two experimental methods was daily changing of the tubes containing the moths as well as the lids (starting from day 3) in this experiment. The extra disturbance caused by moving the moths to a new tube every day might have somehow prevented them from producing super-eggs, but the most likely explanation is that there was some form of cross-contamination between the different days' egg-laying in the previous experiments. The assumption in the pilot experiment and experiment 2 was that the moths would not lay eggs onto the sides of the tubes containing them, but that all of the eggs would collect in the bottom of the apparatus. When I noticed eggs on the sides of the tubes in this experiment, I realised that this was obviously not the case, and that these eggs could hatch and get into later days egg batches where they would appear to have developed more quickly. With hindsight, this was perhaps an error that should have been spotted earlier. However, at the time, the comparison with the egg-machines used for stock culturing had led me to believe that simply collecting eggs in the base of the apparatus would be adequate, and that levels of cross-contamination between days would have been too low to be significant.

A close reading of Sarah Lindfield's thesis suggests that cross-contamination may also explain her results, though her description of how she collected eggs for each of her experiments is rather vague. It implies that for her experiments involving groups of moths (where she found super-eggs) she did not change the container the moths were in, while in her single pair experiment (where she found no evidence of super-eggs) she moved the moths to a new container every day.

The difference in egg laying pattern between the two treatments, though barely significant, confirms the impression gained from the previous two experiments that the moths change their egg laying behaviour in the absence of a suitable substrate. Although perhaps not surprising, this result does have implications for the use of egg-machines in maintaining the stock cultures. Collecting eggs in this way makes it possible to set up standardised densities of moths in stock culture, but at the same time it may also be

selecting for moths that are prepared to lay eggs under adverse conditions. This may be biasing the genetics and behaviour of the lab culture away from the normal, wild type response.

6.5 Experiment 4: egg hatching times

6.5.1 Introduction

This final experiment sought to clear up the conflicting results produced by the previous experiments by looking at egg hatching times. Sarah Lindfield had found a difference in development rate between the offspring of young and old mothers, no matter what stage of development she looked at — she even found a change in egg hatching times with maternal age. This experiment was an attempt to reproduce this egg hatching effect, and it also used different group sizes of moths to check her finding that the maternal effect disappeared when looking at single pairs of moths.

6.5.2 Method

Moths were reared from stock, sexed, and allowed to emerge individually as in the previous experiments. They were then randomly assigned to one of three treatments: 1 pair; 5 pairs; or 10 pairs. The treatments were set up in 250ml plastic fizzy drinks bottles standing on their lids. The moths were left to mate for 24 hours, then every 12 hours subsequently until day 7 they were moved to a new bottle with a new lid (this was done in a 4 C cold room to slow down the moths so that they could be handled more easily). A sample of the eggs that had collected in the old lid were set up for hatching time monitoring by putting them onto the sticky side of an address label in a small petri-dish, using a fine brush (see section 1.2.5). The eggs from each bottle, up to a maximum of 25, were set up on a label in this way, and then each label was examined under a microscope every 12 hours to see how many eggs had hatched. Eggs that had hatched became transparent, and were marked with a fine-tipped black pen to distinguish them from newly hatched eggs in later sampling periods.

6.5.3 Results

None of the "5 pairs" treatments laid any eggs, however 9 of the "single pair" and 11 of the "10 pairs" treatments laid eggs during the experiment. There was a very low hatching success in the eggs that were set up (259 hatched out of a total of 2778 eggs), however the range of hatching times in those that did hatch was very restricted with 84.6 %

hatching 4.5 days after laying and the remainder hatching 12 hours either side of this time. There was no significant difference in hatching time between the treatments (independent samples t-test, $t = -1.662$, d.f. $= 257$, $p = 0.098$). There was also no significant effect of age of mother on hatching time (Figure 6.12).

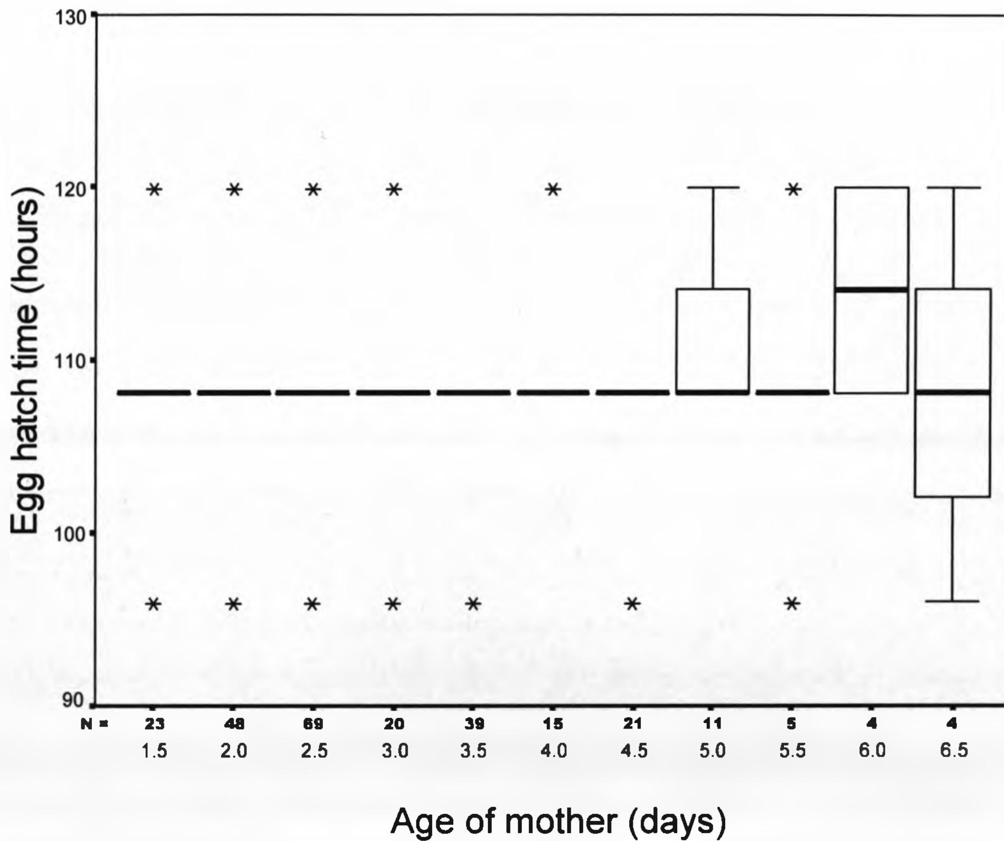


Figure 6.12 Boxplot of egg hatching time for different age mothers from experiment 3.

6.5.4 Discussion

Once again, this experiment gave no evidence for the existence of super-eggs. Indeed, the hatching times of the eggs were remarkably accurate, with all eggs hatching within a 12 hours of 4 ½ days after laying. It is possible that any super-eggs that were laid were among the majority of eggs that did not hatch. However, the hatching time of 4-5 days corresponds very well with the first appearance of 5th instars at days 5-6 (4-5 days after the first eggs were laid) in the previous experiments. This strongly suggests that the apparent change in development rate found in experiments 1 and 2 was caused by eggs from earlier days' laying, hatching and contaminating later days' egg collection.

So the answer to the question posed in the title of this chapter — 'do *Plodia interpunctella* lay "super-eggs"?' — is 'probably not'. The most parsimonious explanation of the results from these four experiments is that my initial findings of "super-eggs" were in fact only the result of cross-contamination caused by my experimental methods — when I refined the methods the effect disappeared. It could also be that under certain conditions moths will lay "super-eggs", but that the change in these experimental methods somehow prevented them from doing so. However, this explanation seems rather tortuous and unlikely.

As yet, there have been very few studies of maternal age on the development of offspring in insects (Mousseau and Dingle, 1991; Fox, 1993a) and those that do exist show conflicting results. Theory, too, differs over the optimal allocation of reproductive effort as a parent gets older (Begon and Parker, 1986; McNamara and Houston, 1996). Although from this study I have no evidence for a maternal age effect in *P. interpunctella*, this does not mean that there was not a more subtle effect that was missed in my experiment or that the effect does not occur in other species. The study of maternal effects is still a relatively unexplored area, yet it is interesting, dealing, as it does, with natural selection acting across generations.

7. Complementary sex determination in *Diadegma chrysostictos*

7.1 Introduction

The original aim of this PhD study was to set up population cages containing one host and two parasitoid species in order to study the long-term population dynamics of such a three-species interaction. Although we had the host — *Plodia interpunctella* — and one of the parasitoid species — *Venturia canescens* — already at Liverpool, I still had to find a suitable second parasitoid species to use. It eventually became clear that I would not be able to find and develop a suitable second parasitoid species quickly, so I switched to doing the work described in the other chapters of this thesis. However, one of the species that I tried out for use in the population cages — *Diadegma chrysostictos* — provided some interesting results during preliminary work, which I will describe in this chapter.

7.1.1 The Problem

In November 1993 I received a culture of a *Diadegma* species from Dr. G. Marris at the Central Science Laboratories in Slough who had collected the original animals in contaminated bran from a nearby grain-store during the previous summer. The species was later identified as *Diadegma chrysostictos* (Gmelin) by Dr. M. Shaw at the National Museum of Scotland, Edinburgh. Of the original pupae that were sent to me, only 3 females and 7 males emerged successfully, so I had to establish cultures from very few founders. In the succeeding generations of wasps that emerged, there was an extremely male-biased sex ratio (approximately 80–95% males) which did not seem to be affected by rearing at different temperatures (I tried 20, 25 and 28 C), or by allowing the females to parasitize at different relative densities of male and female wasps or on different host species.

Eventually, after 4 generations, the culture died out when no females emerged at all, but before this happened, I had already done some tests to try to find out what was causing such a highly skewed sex-ratio. In the rest of this chapter, I shall first look at the different

ways in which sex ratio can be affected in the Hymenoptera, and then describe the results of my own work on *D. chrysostictos*.

7.2 Sex ratio in Hymenoptera

The Hymenoptera display a huge variety of sexual and social strategies, prompting many scientists to use them for testing theories about optimum sex allocation strategies, so there is a large body of knowledge on the factors affecting sex ratio in the group. A very extensive and recent review of sex ratio in Hymenoptera, and parasitoids in particular, can be found in Godfray (1994), but I will summarize and expand upon the relevant areas here.

The eventual sex ratio of the offspring of a hymenopteran female can depend on a variety of internal and external factors. External factors, such as population density and composition, host species and quality, cannibalism and environmental conditions will affect the sex ratio strategy that the female tries to adopt, and the subsequent survival of her offspring. Internal factors, such as the sex determination system and the amount and viability of stored sperm, affect the female's ability to manipulate the sex ratio of the eggs as she lays them.

7.2.1 External factors affecting sex ratio

7.2.1.1 Population density and sex ratio

A female may vary the sex ratio of her offspring in response to changes in the density and sex ratio of other conspecifics that she encounters. By doing so, she may be able to improve the survival of her offspring and their ability to mate successfully. This is a very well studied area — encompassing theories of local mate competition and optimum sex ratio allocation — that is too large to go into any detail here, see Hardy (1994) for a recent review.

7.2.1.2 Sibling cannibalism

In situations where eggs are laid at high densities, it may be possible for one sex to hatch earlier and eat the eggs of the other sex before they can hatch, thus affecting the sex ratio of the surviving offspring. Rotary and Gerling (1973) found that male progeny of *Bracon hebetor* emerged before females and suspected that subsequent cannibalism led to a male biased sex ratio. Benson (1973) found that at high densities of *B. hebetor* eggs (18 per

host), 80% of the surviving offspring were male, while at low densities (3 per host), the sex ratio was 50% male. He also suggested that this might be due to male eggs hatching earlier, and at high densities eating the unhatched, female eggs, causing a sex ratio bias, however Taylor (1988) found no such effect on sex ratio.

7.2.1.3 Sperm depletion

Females may start laying male biased sex ratios if they run out of stored sperm or if the sperm is not viable for some reason. Antolin and Strand (1992) found that in a field population of *Bracon hebetor*, older females were more likely to have empty spermathecae even though they were still able to lay eggs, while females will often run out of sperm in laboratory situations (King, 1987). The sperm stored by a female may sometimes be infertile and so she will be unable to lay fertilised eggs. This can occur if the male that she mates with is old or has experienced extremes of temperature (King, 1987), or if he is a diploid male (Cook and Crozier, 1995, see below).

7.2.2 Sex determination systems

7.2.2.1 Haplo-diploidy

The basic hymenopteran form of sex determination is haplo-diploidy, where an unfertilized (haploid) egg develops into a male and a fertilised (diploid) egg becomes a female. This allows a hymenopteran female to manipulate the sex ratio of her offspring simply by controlling the access of sperm to the eggs as she lays them. As knowledge of the Hymenoptera improves, however, discoveries of alternative or complementary sex determination systems are clouding this initially simple picture. Diploid and even triploid males have been found in several species that come from widely spread taxonomic groups within the Hymenoptera. The genetic and molecular mechanism which underlies haplo-diploidy is still poorly described and this has hindered understanding of the evolution of haplo-diploidy and these other sex determination systems.

7.2.2.2 Complementary sex determination (CSD)

There are a number of Hymenoptera species that will produce diploid males as well as the normal haploid variety. These diploid males are usually infertile —although fertile diploid males have been found in a sawfly species (Naito and Suzuki, 1991) — and their appearance is often associated with inbreeding. This phenomenon has given rise to the

theory of complementary sex determination (CSD), which in its simplest form supposes the existence of a gene with several alleles that controls the sex of offspring (Cook, 1993b; Cook *et al.*, 1994). If the CSD gene is heterozygous in a fertilised egg then that egg will develop into a female, but if the gene is homozygous then the egg will produce a diploid male. With this system, genetic drift in a small, inbred population would reduce the number of different alleles in the population, increasing the likelihood that a female would mate with a male carrying the same CSD allele as one of her own, and so leading to the production of diploid males.

Diploid males have been discovered in several species from different groups within the Hymenoptera — including bees, ants (Ross and Fletcher, 1985), sawflies (Naito and Suzuki, 1991) and several parasitoids (Periquet *et al.*, 1993; Cook and Crozier, 1995). The system has been studied most closely, however, in a braconid parasitoid, *Bracon hebetor*. CSD in *B. hebetor* is controlled by a single gene with at least nine alleles (see Figure 7.1) (Whiting, 1943). If a diploid (i.e. normally female) zygote has the same allele (homozygous) for both copies of the gene, then the egg will develop into a diploid male, while a heterozygous egg will develop into a normal female. Haploid zygotes still develop as normal males. This system means that if the father has the same allele for the CSD gene as one of the mother's then a proportion of the offspring will be diploid males. Most *B. hebetor* diploid males die either as eggs or as early larvae, though in some strains a proportion do survive to adulthood.

Inbreeding tends to increase homozygosity, and so increases the proportion of diploid males in a population, causing unpredictable fluctuations in population sex ratio. Perversely, in *B. hebetor*, extreme inbreeding — when a population is established from the offspring of a single mating — can remove the sex ratio effects of CSD altogether, by ensuring that only two CSD alleles are present. In such a population, all matings are like the example in Figure 7.1(b), leading to a constant sex ratio of about 1:1 (Cook *et al.*, 1994).

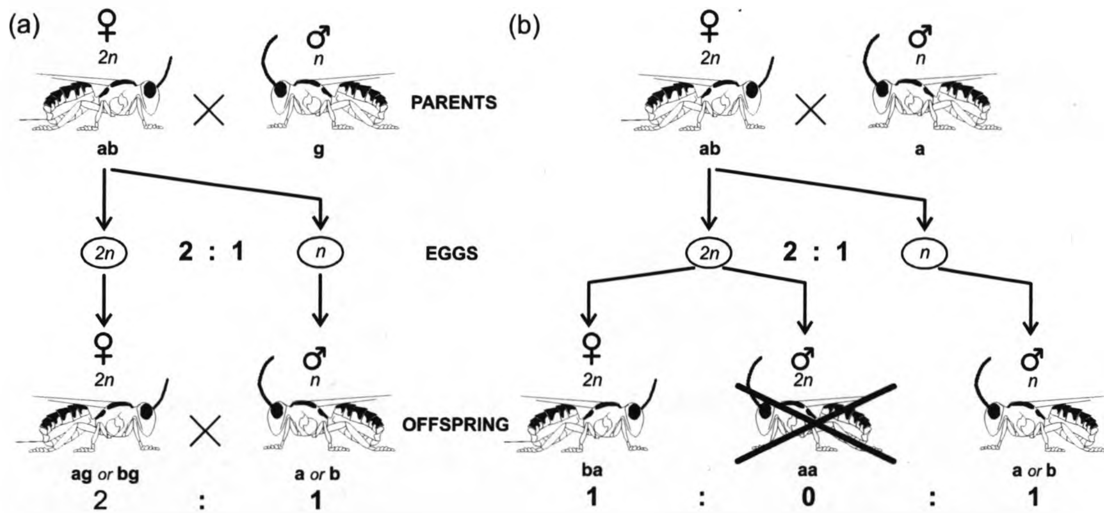


Figure 7.1. The complementary sex determination (CSD) system of *Bracon hebetor* involves a single-locus gene, with nine alleles. **(a)** If the parents have all different alleles for the CSD gene (a,b,g), then all diploid (2n) offspring are heterozygous and female, while haploid (n) offspring are male, as in the normal hymenopteran haplo-diploid system. Females tend to lay twice as many fertilised as unfertilised eggs, so the sex-ratio of the adult offspring will be 2:1 in favour of females. **(b)** If the father has the same CSD allele as one of the female's, then half of the diploid offspring will be homozygous. These homozygotes become diploid males, which usually die during development, leaving an adult sex-ratio of 1:1.

Evidence of CSD has so far been shown in at least 33 species from a wide range of different groups within the Hymenoptera (Cook and Crozier, 1995), however there are also some species in which it has been shown that CSD is not present (Cook, 1993a; Orzacht and Gladstone, 1994). As more and more Hymenoptera species are shown to have some form of CSD, it has been suggested that CSD is really the ancestral form of sex determination in this group (Cook, 1993a). Bull (1981) uses a model to show how haplo-diploidy might evolve from an ancestral form in which both males and females are diploid, but sex is determined by hetero- or homozygosity at a sex locus. His model relied on some selective advantage for males being haploid, and intriguingly such an advantage has recently been discovered in the "functional" haplo-diploidy of the coffee berry borer beetle, *Hypothenemus hampei* (Brun *et al.*, 1995a; Brun *et al.*, 1995b). This species is functionally haplo-diploid because the paternal chromasomes become degenerate in males and are not passed on in sperm (Brun *et al.*, 1995a). This feature, combined with a mating system that leads to extreme inbreeding, has allowed the rapid spread of insecticide resistance in *H. hampei* populations through efficient maternal inheritance of resistance genes (Brun *et al.*, 1995b).

7.2.2.3 Detecting CSD

Although an increase in the proportion of males in inbred populations is a good indicator of CSD, there are several techniques that can give a more positive identification. These can include morphometric studies (Grosch, 1945; Ross and Fletcher, 1985), the use of phenotypic markers in crossing experiments (Naito and Suzuki, 1991; Periquet *et al.*, 1993) and electrophoresis using genetic markers (Ross and Fletcher, 1985) to detect diploid males.

7.2.3 *Diadegma chrysostictos*

Diadegma chrysostictos is an Ichneumonid, solitary endo-parasitoid wasp, that parasitises a variety of Lepidopteran larvae (Fisher, 1959; Horstmann and Shaw, 1984). It has been used in relatively little experimental work to date, the most important studies being those of Fisher (1959; 1961b; 1961a; 1962). Fisher attempted to set up long-term populations of *Diadegma* (then called *Horogenes*) *chrysostictos* together with another parasitoid, *Venturia canescens*, with *Ephestia kuehniella* as the host. He found, however, that in both the experimental and control populations, *D. chrysostictos* showed an increasingly male biased sex ratio, and died out within 14 weeks (Fisher, 1962). In the current study, I decided to test the possibility that *D. chrysostictos* showed CSD by trying to detect diploid males in my inbred populations, using a morphometric technique. This technique relies on the observation that diploids tend to have larger cell sizes than haploids, and this is most easily seen by measuring the density of microchaetae on the surface of the wing, as each wing cell produces one microchaeta (Grosch, 1945).

7.3 Method

The data were collected from 2 pairs of sub-populations set up in succeeding generations. Each sub-population was set up by placing 5 male and 5 female, recently emerged *Diadegma chrysostictos* adults in a plastic box, together with about 100 final instar hosts in food medium. The hosts for the first two sub-populations were *Plodia interpunctella* in one case and *Ephestia* (*Anagasta*) *kuehniella* in the other. The second two sub-populations both used *P. interpunctella*. In all cases, the hosts were reared on the same food medium and in the same conditions as the 25°C *P. interpunctella* cultures (see chapter 1). Once all of the wasps had emerged in a sub-population, the males were removed and quickly killed by freezing, before the right forewing of each male was removed and mounted on a microscope slide. The wings were examined using a binocular microscope fitted with a

squared eyepiece graticule for the first two sub-populations and with a video microscope for the second two sub-populations. For each wing, the number of bristles (microchaetae) was counted within a known area on a section of membrane between the radial sector and media veins (see Figure 7.2) and the length of the media and media+media-cubitus veins was measured in order to compensate for size variations.

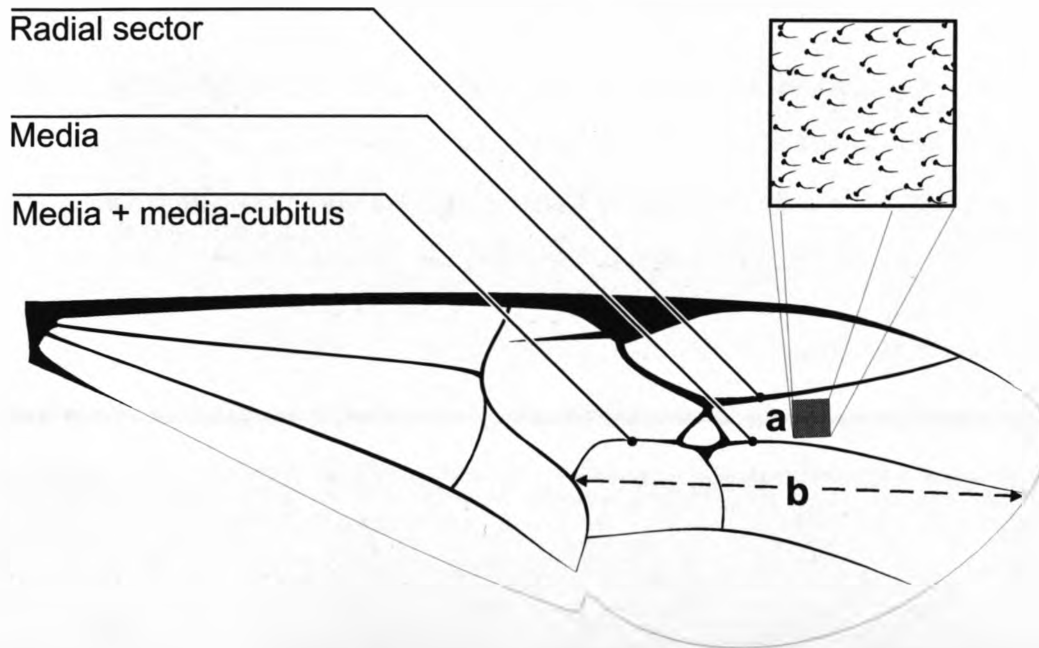
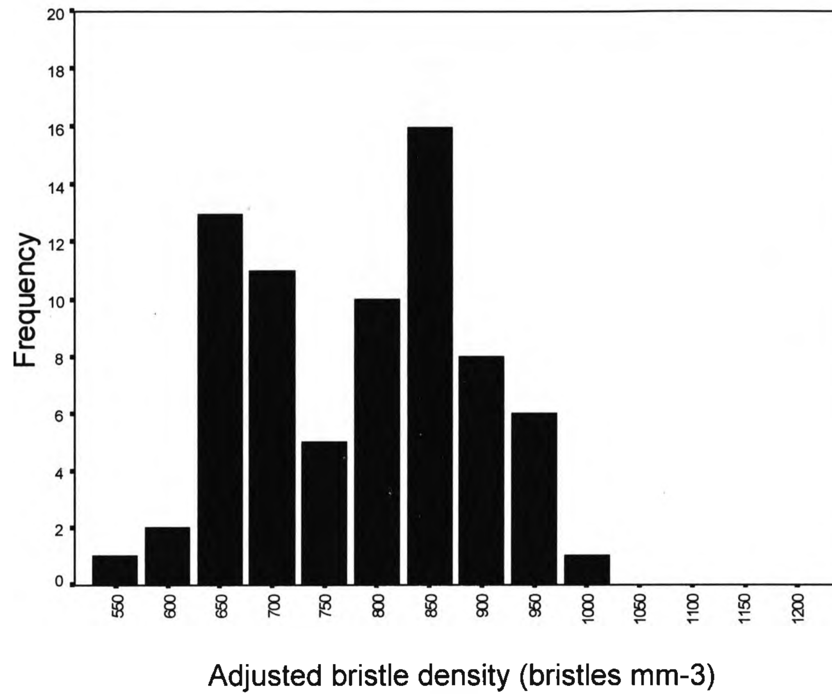


Figure 7.2. A diagram of the right forewing of *Diadegma chrysostictos*, showing the region (a) in which the bristle density was measured, and the measurement (b) used for size comparisons.

7.4 Results

The adjusted wing bristle densities (bristles per mm^2 per mm of wing vein length) of males from the 4 sub-populations are shown in Figure 7.3. The frequencies from the first two sub-populations (Figure 7.3a) show two peaks at densities of about 650 and 850 bristles mm^{-3} while in contrast, the second two sub-populations (Figure 7.3b) show only one peak at about 900 bristles mm^{-3}.

(a)



(b)

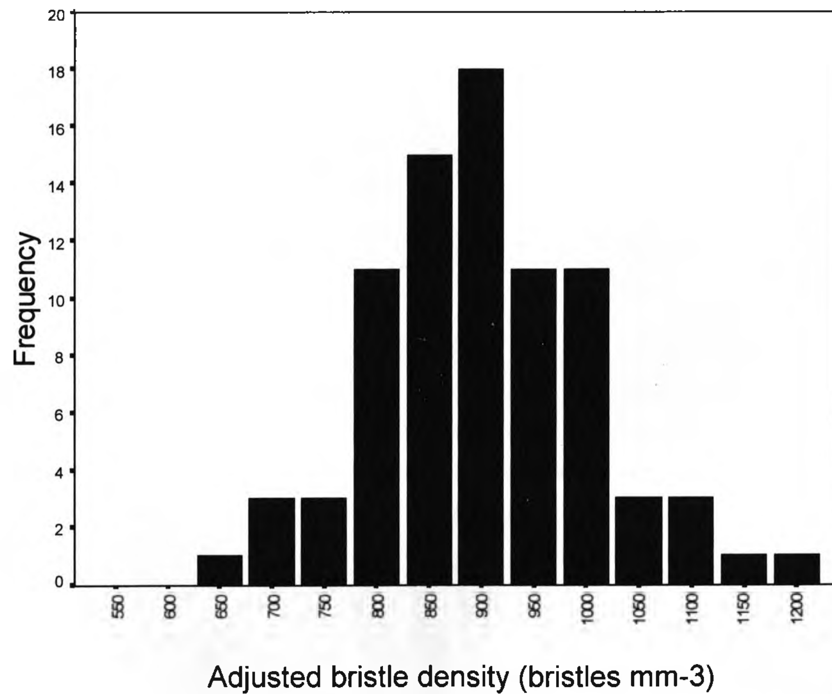


Figure 7.3 Frequency histograms for *D. chrysostictos* wing bristle density (adjusted for body size). (a) sub-populations 1 and 2 combined; (b) sub-populations 3 and 4 combined.

7.5 Discussion

The data from this experiment are rather inconclusive. If diploid males are present in a population then one would expect a graph of frequency against wing bristle density to show two peaks, the upper one for haploids and the lower one for diploids (Grosch, 1945). This appears to be the case in the first two sub-populations but not from the second two, where there is only one peak, roughly corresponding to the upper, haploid peak in the first. This situation could have occurred if the females in the second two sub-populations had not mated or only mated with sterile, diploid males. Such females would only lay haploid male eggs, giving rise to an all male offspring sex ratio. This is supported by the fact that no females emerged at all from the second two sub-populations, whereas if the founding females had successfully mated a fertile male one would expect at least a small proportion of females in the offspring. In my case, the culture of *Diadegma chrysostictos* was fast becoming extinct, so I was unable to do follow up studies to confirm this.

While not offering conclusive evidence of diploid males in *D. chrysostictos*, this study suggests that further investigations may show the presence of complementary sex-determination in this species. Indeed, such evidence has recently been found in other strains of the same species by Robert Butcher at the University of Dundee using protein and DNA analysis techniques. The system appears to be a complex one involving sex-determination at two or more loci with several alleles (R. Butcher, pers. comm.).

The testing of CSD in more species is essential before the nature and evolution of sex determination systems can be fully understood in the Hymenoptera (Cook, 1993b). There are several theories relating to the evolution of sex determination in the Hymenoptera (Bull, 1981; Cook and Crozier, 1995), but as yet, there are few definitive experimental tests.

CSD is theoretically interesting, but can pose serious problems in attempts to mass rear parasitoids in a laboratory or for biological control attempts. Steps must be taken to ensure that an adequate number of specimens are collected from the wild, and to avoid inbreeding in subsequent generations in order to maintain enough different alleles at the sex locus (Stouthamer *et al.*, 1992). This will be a particular problem if the diploid males survive into adulthood, as appears to be the case in *D. chrysostictos*, since they will mate with females who will then not produce any fertilised eggs. Thus the technique of

extreme inbreeding that is effective for *Bracon hebetor* (Cook *et al.*, 1994) will not work here. CSD also has important implications for experimental studies on other aspects of sex ratio theory in the Hymenoptera, since if it is not taken into account, it may invalidate any sex ratio data that is collected (Owen and Packer, 1994). The technique of wing bristle density measurement used here may provide an easy method to test for the presence of diploid males in a sample of parasitoids, provided that a reasonable number of individuals are available.

8. General Discussion

8.1 Introduction

At the beginning of this thesis I said that it was difficult to draw the different bodies of work described here under one banner for the purpose of a general introduction. This could also be true of a general discussion. In this final chapter I will summarise the results from the previous chapters before going on to discuss some of these results in the light of population studies of *Plodia interpunctella*.

8.2 Summary of results

In chapter 2 I described two experiments looking at the possible effects of parasitism on host cannibalism. The first experiment showed that in individual encounters between parasitised and unparasitised larvae, the parasitised hosts were cannibalised more often. The second experiment did not show this differential cannibalism in groups of larvae at different densities in food, but the results may also have been affected by disease in the later stages of the experiment.

The differential cannibalism found in chapter 2 held interesting possibilities for the population dynamics of host-parasitoid systems. In chapter 3 I explored these possibilities, and the more general effects of host cannibalism, using variations of a Lotka-Volterra type model of host-parasitoid population dynamics. The general consequences of cannibalism in these models were (1) increased stability, (2) reduction of equilibrium parasitoid densities with a corresponding increase in host density, and (3) a smaller region of parameter space where the parasitoid could persist. These features would probably make cannibalism an advantage for the host, in the presence of parasitism.

Chapter 4 used a factorial experiment with different initial numbers of eggs and amounts of food, to explore the effects of density on cohorts of *Plodia interpunctella*. There were strong effects of density on egg-to-adult survival, and on adult characters such as size and reproductive potential. Survival and adult size showed thresholds in their response to density, with no effect below a density of about 9 eggs per g food. Ovary and testis size

showed strong allometry with body size, which was, in turn affected by density. However, any extra effects of density on reproductive investment were very weak and negative.

The work in chapter 5 followed on from that in the previous chapter by focusing on the development of the larvae, to see when density had an effect. In cohorts of *P. interpunctella* larvae, higher densities lead to increased mortality and development time in the 4th and 5th instars. Much of this mortality was caused by cannibalism between the later instars, which also cannibalised pupae. The effects of density were also felt in the adult stage, with smaller adult size and shorter life span. I compared two different methods for analysing the stage-frequency data to find the development times and mortalities for each stage. The method that allowed mortality rates to vary with time provided a better description of the results.

In chapter 6, I followed up the work of a previous student at Liverpool, who found that older adults of *P. interpunctella* laid eggs that developed faster. Through a set of successively more refined experiments, I showed that these apparent "super-eggs" were probably an artefact of experimental technique, with eggs from previous days laying contaminating later batches.

Finally, in chapter 7, I described evidence for a complementary sex determination system in *Diadegma chrysostictos*, another parasitoid of *P. interpunctella* that I was hoping to use for population studies. The high ratio of males to females suggested that diploid males were being produced, and measurements of wing cell size seemed to confirm this.

8.3 Discussion

Long-term studies of single species and multiple species systems have contributed enormously to the understanding of population dynamics processes. Theoretical methods distil hypotheses about population processes into mathematical models that can be tested against these long-term data sets. Several long-term data sets on the population dynamics of *P. interpunctella* and of its natural enemies have been collected at Liverpool (Sait *et al.*, 1994; Begon *et al.*, 1996; Sait *et al.*, 1998). Much of the work in this thesis was aimed at using short-term experiments to help explain the behaviour of these data sets.

It is hard to collect detailed information on interactions between individuals within a laboratory population without disturbing the dynamics of the system. Therefore, the

populations run by Sait were, to some extent, "black boxes". The general behaviour of the populations could be recorded, but the underlying processes generating that behaviour could not be observed directly. Theoretical modelling can suggest possible mechanisms for population fluctuations, but these models need accurate estimates for their parameters, and their assumptions have to be tested by experiment. In this discussion I will compare my results with the assumptions made by recent models of *P. interpunctella* population dynamics.

The difficulty with designing experiments to investigate the conditions within long-term populations is a conflict between realism and analytical power. It is usually impossible to recreate fully the conditions within a population and at the same time pick out and focus on the individual factors responsible for those conditions. The experiments described in this thesis were gross simplifications of the already simplified population cages, yet I believe that they can still be useful in interpreting the population data.

The experiments described in chapters 4 and 5 followed the development of cohorts at different initial densities from eggs through to adults. These provided some important insights for attempts at modelling *P. interpunctella* populations: firstly, the effects of density were not apparent in the earlier larval instars, effectively dividing development into density-insensitive, and density-sensitive stages; secondly, the seemingly inert pupal stage was strongly affected by density-dependent cannibalism by larvae; and thirdly, the effects of density extended into the adult stage, altering size and life-span, and even into the next generation through changes in adult reproductive potential. The onset of density-dependence was not a smooth transition as density increased, but rather there were thresholds in the response to density.

Some of these findings confirm assumptions that have already been incorporated into recent models of *P. interpunctella* population dynamics. Bjørnstad *et al.* (1998) divided the larval stage into "young" and "old" classes that had different responses to density, and this sort of division is supported by my experiments. However, this model also allows for competitive and cannibalistic interactions between these age classes and for both age classes to cannibalise eggs. This points out a limitation in my experiments: by following a single cohort of larvae these sorts of interactions between stages were not really possible. In a real population there will be a mixture of different stages, and interactions between them will be important for the dynamics of the population (Hastings and Costantino,

1987; Loreau, 1990; Hastings and Costantino, 1991; Nisbet and Onyiah, 1994). Experiments to explore the interactions between stages, while much more complex, would be the next logical step in trying to understand the *P. interpunctella* population data.

Some of the other assumptions of the Bjørnstad *et al.* model are not supported by my experiments. They assume that there is no effect of density on pupal mortality, yet the experiment in chapter 5 showed a clear, density-dependent pupal mortality. They also assume that the durations of each stage will not be affected by density, yet my experiment showed that the duration of "old" larvae (instars 4 and 5) increased with density. Finally, they assume that the reproduction by adults is not affected by the density they experienced as larvae, but the experiment in chapter 4 showed that density had a large effect on adult reproductive potential. Some of these factors are being incorporated into a new model of *P. interpunctella* population dynamics, developed by Briggs et al. (*in prep.*). This will include density-dependent pupal mortality, and the new estimate of the duration of the late larval stage provided by my experiments.

The threshold in the response of mortality and size to density suggested that the larvae were competing in a 'scramble' fashion (Begon *et al.*, 1990; Toquenaga, 1990). This will have a destabilising effect on the population dynamics of *P. interpunctella* (Smith and Lessells, 1985), and should be considered in future population models. The fact that previous data sets suggested that competition in *P. interpunctella* was only 'scramble-like' (Begon *et al.*, 1990) demonstrates the importance of exploring the widest possible range of densities in competition experiments (Smith and Lessells, 1985; Credland *et al.*, 1986).

The importance of the adult stage is often neglected in models of stored product pest population dynamics. A convenient assumption is that adults lay a constant *per capita* number of eggs as soon as they emerge, with no effect of density (Lynch *et al.*, 1998). Yet my experiments show that this is clearly not true. Reproductive potential in *P. interpunctella* is heavily density-dependent, and this will probably have important consequences for population dynamics (Hassell, 1975).

The cannibalism experiments in chapter 2, and the subsequent modelling work in chapter 3, showed that it is also important to consider the possible effects of host density on parasitoids. The parasitoid may respond to density in a different way to the host, and this could have fundamental effects on the dynamics of a host-parasitoid system. This may

even be responsible for the evolution of cannibalism where it might otherwise be selected against. The Lotka-Volterra type modelling approach in chapter 3 was relatively simplistic in its structure and assumptions. While this approach may still be valuable and analytically powerful (Lynch *et al.*, 1998), more complex, stage-structured approaches like those described above may be more appropriate for future attempts at modelling cannibalism in *P. interpunctella* populations.

As already mentioned, there were significant limitations in the experiments in chapters 4 and 5: using single cohorts of larvae meant that there were not really any interactions between different developmental stages; and the food was not replenished during the experiments, possibly exaggerating the effects of density as the food was used up. These limitations mean that the results may not be directly comparable to the long-term population data. Although the processes shown by my experiments probably apply in the populations, their magnitudes and timing may well be different. Nonetheless, my results, and the parameter estimates gained from them, come a step closer to understanding the real factors at work in populations of *P. interpunctella*.

Further experiments need to recreate the conditions of the populations more closely, including using mixtures of different ages and stages of individuals, replenishing food, and working on a larger scale. An example might be to do repeats of the experiment in chapter 5, but rather than following a cohort through its complete life-span, concentrate on specific instars, both on their own and in mixtures with other instars (and eggs and pupae). This will give an idea of the amounts of cannibalism between instars and stages in a mixed population, and will also reveal the 'competitive effect' of different densities of one stage or instar on another. Another experiment might be similar to that in chapter 4, but replenishing or adding food at set intervals — as in the population cages — to see what effect this might have on levels of competition and density-dependence.

Once the experiments suggested above have been done, the information gained could be used to examine the effects of host competition on a second species, such as a parasitoid or pathogen, in much more detail. A repeat of the second experiment in chapter 2, for example, could focus on known specific density-dependent and density-independent larval densities and examine the effects of parasitism by *V. canescens* on levels of cannibalism and competition. The experimental work could also lead to new theoretical avenues to explore as well. These might include extensions of existing models to take

account of the competitive effects that I have found here, as well as the use of newer techniques, such as delay-differential and individual-based models, which are better able to cope with the complex interactions between and within stages.

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Appendix A

This is a listing of the Microsoft Excel 95 Visual Basic program used for the analysis described in section 5.4.3.

```
' This is a method for analysing stage-frequency data taken from
' Manly, B.F.J. (1993) Res. Popul. Ecol. 35:215-222.
'
' The macro was written by Douglas J. Reed in May 1998
'
' The procedures reference two other worksheets: the "Calculation Sheet" holds the
' initial values to use for the parameters and displays the results. "Complete Counts"
' holds all of the stage-frequency counts for the analysis.

' Ensures that variable names are checked for errors, helping to trap bugs
Option Explicit

' These are the global variables required by all of the procedures in the program.
' Their names mostly correspond to the parameters in the model description, so should
' be fairly self-explanatory.

Dim PhiMin, PhiMax, PhiStep, Phi, SDPhi As Double
Dim RepPhis(10), RepAlphas(8, 10), Alpha(8) As Double
Dim f(60, 8), fHat(60, 8), Meanf(60, 8) As Double
Dim StartDay, EndDay, StartStage, EndStage, RepNumber, CurrentRep, Treatment As Integer
Dim Sigmafji(8), Sigmafji_m(8), Sigmafj_mi_m(8) As Double


Sub ManlyMainRoutine()

' Loops through the replicates of a treatment, analysing each one and
' then obtaining mean values and standard errors. This Macro is called
' by the Run button on the Calculation Sheet

  Dim RepCount As Integer
  GetSetupValues
  For RepCount = 1 To RepNumber
    CurrentRep = RepCount
    GetRepValues
    FindBestSDPhi
    StoreRepData
  Next RepCount
  SummariseData
End Sub


' ***** ManlyMainRoutine Procedures *****

Private Sub GetSetupValues()

' Gets the initial parameters for the run from the Calculation Sheet

  Dim i, j, k As Integer
  Phi = Worksheets("Calculation Sheet").Range("B5").Value
```

```
  PhiMin = Worksheets("Calculation Sheet").Range("B6").Value
  PhiMax = Worksheets("Calculation Sheet").Range("B7").Value
  PhiStep = Worksheets("Calculation Sheet").Range("B8").Value
  Treatment = Worksheets("Calculation Sheet").Range("B3").Value
  StartDay = Worksheets("Calculation Sheet").Range("B10").Value
  EndDay = Worksheets("Calculation Sheet").Range("B11").Value
  StartStage = Worksheets("Calculation Sheet").Range("B13").Value + 1
  EndStage = Worksheets("Calculation Sheet").Range("B14").Value + 1
  RepNumber = Worksheets("Calculation Sheet").Range("B16").Value
  For i = 1 To 60
    For j = 1 To 8
      Meanf(i, j) = 0
    Next j
  Next i
  For k = 1 To 10
    RepPhis(k) = 0
    For j = 1 To 8
      RepAlphas(j, k) = 0
    Next j
  Next k
End Sub


Private Sub GetRepValues()

' Gets the parameters for a specific replicate

  Dim CurrentRow, RowOffset, j, i, h, s As Integer
  Dim FoundData As Boolean
  Worksheets("Calculation Sheet").Range("F7:H13").Clear
  Worksheets("Calculation Sheet").Range("G2").Value = CurrentRep
  RowOffset = (((Treatment / 4) - 1) * 600) + ((CurrentRep - 1) * 60) + 1
  EndDay = Worksheets("Calculation Sheet").Range("B11").Value
  EndStage = Worksheets("Calculation Sheet").Range("B14").Value + 1

  ' Find the last row to contain data

  FoundData = False
  h = EndDay + 1
  Do
    h = h - 1
    For j = StartStage To EndStage
      If Worksheets("Complete Counts").Cells(RowOffset + h, j + 2).Value > 0 _
        Then FoundData = True
    Next j
  Loop Until FoundData
  EndDay = h

  ' Find the last Stage to contain data
  s = EndStage + 1
  Do
    s = s - 1
    h = EndDay + 1
    Do
      h = h - 1
    Loop Until Worksheets("Complete Counts").Cells(RowOffset + h, s + 2).Value > 0
  Loop Until h > 0
  EndStage = s
  Worksheets("Calculation Sheet").Range("H2").Value = EndDay
  Worksheets("Calculation Sheet").Range("I2").Value = EndStage - 1

  ' Fill the values of f(i,j) with the values from the Complete Counts sheet
```

```
  For i = StartDay To EndDay
    CurrentRow = i + RowOffset
    For j = StartStage To EndStage
      f(i, j) = Worksheets("Complete Counts").Cells(CurrentRow, j + 2).Value
      Meanf(i, j) = Meanf(i, j) + f(i, j)
    Next j
  Next i

  ' The section below calculates the various Sums of f(i, j) required for the main formula.
  ' It is only necessary to calculate these once for each dataset
  Alpha(StartStage) = 0
  For j = StartStage + 1 To EndStage
    Alpha(j) = 0
    Sigmafji(j) = 0
    Sigmafji_m(j) = 0
    Sigmafj_mi_m(j) = 0
    For i = StartDay + 1 To EndDay
      Sigmafji(j) = Sigmafji(j) + f(i, j)
      Sigmafji_m(j) = Sigmafji_m(j) + f(i - 1, j)
      Sigmafj_mi_m(j) = Sigmafj_mi_m(j) + f(i - 1, j - 1)
    Next i
  Next j
End Sub



Private Sub FindBestSDPhi()

' This is the iteration routine that finds the value of Phi that best fits the data

  Dim p, BestSD, BestPhi, PMin, PMax, PStep As Double
  BestSD = 10000
  BestPhi = Phi
  PMin = PhiMin
  PMax = PhiMax
  PStep = PhiStep
  Do

    ' Step through a range of values of Phi and test each one
    For p = PMin To PMax Step PStep
      Phi = p
      Worksheets("Calculation Sheet").Range("F3").Value = Phi
      CalculateAlphas
      CalculateExpecteds
      CalculateSDPhi
      If SDPhi < BestSD Then
        BestPhi = Phi
        BestSD = SDPhi
      End If
    Next p

    ' Once the best value of Phi has been found, choose new PMin and PMax values
    ' around the best value, and reduce the step size before repeating the process.
    ' Make sure that that the PMin and PMax values are not outside the 0-1 range.
    PMin = BestPhi - PStep
    If PMin < 0 Then PMin = 0
    PMax = BestPhi + PStep
    If PMax > 1 Then PMax = 1
    PStep = PStep / 10

    ' The value used below determines the final accuracy of the estimate.
  Loop Until PStep < 0.00001
  Phi = BestPhi
```

```
  Worksheets("Calculation Sheet").Range("F3").Value = Phi

  ' Once the best value of Phi has been found, use it to recalculate the estimates of
  ' other parameters, so that they can be stored for later use.
  CalculateAlphas
  CalculateExpecteds
  CalculateSDPhi
End Sub



Private Sub StoreRepData()

' Simply stores the values of the estimates from each replicate in arrays

  Dim j As Integer
  RepPhis(CurrentRep) = Phi
  For j = StartStage To EndStage
    RepAlphas(j, CurrentRep) = Alpha(j)
  Next j
End Sub



Private Sub SummariseData()

' Produces means and standard errors of the estimates from each replicate

  Dim h, i, j, r As Integer
  Dim FoundData As Boolean
  Dim Survivals(8, 10), Durations(8, 10) As Double
  Dim MeanSurvival(8), MeanDuration(8), MeanAlpha(8), MeanPhi As Double
  Dim SSPhi, SSSurvival(8), SSDuration(8), SSAlpha(8) As Double
  Dim SEPhi, SESurvival(8), SEDuration(8), SEAlpha(8) As Double
  EndDay = Worksheets("Calculation Sheet").Range("B11").Value
  EndStage = Worksheets("Calculation Sheet").Range("B14").Value + 1
  MeanPhi = 0
  Worksheets("Calculation Sheet").Range("F24:K32").Value = ""
  For r = 1 To RepNumber
    MeanPhi = MeanPhi + RepPhis(r)
    For j = StartStage To EndStage

      ' Calculate through-stage survival
      Survivals(j, r) = (RepPhis(r) * RepAlphas(j, r)) / _
                        (1 - (RepPhis(r) * (1 - RepAlphas(j, r))))

      ' Calculate mean stage duration
      Durations(j, r) = 1 / (1 - (RepPhis(r) * (1 - RepAlphas(j, r))))
    Next j
  Next r
  MeanPhi = MeanPhi / RepNumber
  Worksheets("Calculation Sheet").Range("F20").Value = MeanPhi
  SSPhi = 0

  ' The next section calculates the Standard Error of the mean of Phi
  For r = 1 To RepNumber
    SSPhi = SSPhi + ((RepPhis(r) - MeanPhi) ^ 2)
  Next r
  SEPhi = Sqr((SSPhi / (RepNumber - 1)) / RepNumber)
  Worksheets("Calculation Sheet").Range("F21").Value = SEPhi

  ' Now calculate means and Standard Errors for Survival, Duration and
  ' Transition rate of each stage.
```

```
For j = StartStage To EndStage
  MeanSurvival(j) = 0
  MeanDuration(j) = 0
  MeanAlpha(j) = 0
  SESurvival(j) = 0
  SEDuration(j) = 0
  SEAlpha(j) = 0
  For r = 1 To RepNumber
    MeanSurvival(j) = MeanSurvival(j) + Survivals(j, r)
    MeanDuration(j) = MeanDuration(j) + Durations(j, r)
    MeanAlpha(j) = MeanAlpha(j) + RepAlphas(j, r)
  Next r
  MeanSurvival(j) = MeanSurvival(j) / RepNumber
  Worksheets("Calculation Sheet").Cells(23 + j, 8).Value = MeanSurvival(j)
  MeanDuration(j) = MeanDuration(j) / RepNumber
  Worksheets("Calculation Sheet").Cells(23 + j, 10).Value = MeanDuration(j)
  MeanAlpha(j) = MeanAlpha(j) / RepNumber
  Worksheets("Calculation Sheet").Cells(23 + j, 6).Value = MeanAlpha(j)
Next j
For j = StartStage To EndStage
  SSSurvival(j) = 0
  SSDuration(j) = 0
  SSAlpha(j) = 0
  For r = 1 To RepNumber
    SSSurvival(j) = SSSurvival(j) + ((Survivals(j, r) - MeanSurvival(j)) ^ 2)
    SSDuration(j) = SSDuration(j) + ((Durations(j, r) - MeanDuration(j)) ^ 2)
    SSAlpha(j) = SSAlpha(j) + ((RepAlphas(j, r) - MeanAlpha(j)) ^ 2)
  Next r
  SESurvival(j) = Sqr((SSSurvival(j) / (RepNumber - 1)) / RepNumber)
  Worksheets("Calculation Sheet").Cells(23 + j, 9).Value = SESurvival(j)
  SEDuration(j) = Sqr((SSDuration(j) / (RepNumber - 1)) / RepNumber)
  Worksheets("Calculation Sheet").Cells(23 + j, 11).Value = SEDuration(j)
  SEAlpha(j) = Sqr((SSAlpha(j) / (RepNumber - 1)) / RepNumber)
  Worksheets("Calculation Sheet").Cells(23 + j, 7).Value = SEAlpha(j)
Next j

' Now calculate the mean values of f(i, j)
Alpha(StartStage) = 0
For j = StartStage To EndStage
  For i = 1 To 60
    Meanf(i, j) = Meanf(i, j) / RepNumber
    f(i, j) = Meanf(i, j)
  Next i
Next j
EndDay = Worksheets("Calculation Sheet").Range("B11").Value
EndStage = Worksheets("Calculation Sheet").Range("B14").Value + 1

' Find the last day to contain data in the Meanf(i, j)s
FoundData = False
h = EndDay + 1
Do
  h = h - 1
  For j = StartStage To EndStage
    If Meanf(h, j) > 0 _
      Then FoundData = True
  Next j
Loop Until FoundData
EndDay = h

' Recalculate the various sums needed for the equations from the mean data
For j = StartStage + 1 To EndStage
  Alpha(j) = 0
  Sigmafji(j) = 0
```

```
    Sigmafji_m(j) = 0
    Sigmafj_mi_m(j) = 0
    For i = StartDay + 1 To EndDay
      Sigmafji(j) = Sigmafji(j) + Meanf(i, j)
      Sigmafji_m(j) = Sigmafji_m(j) + Meanf(i - 1, j)
      Sigmafj_mi_m(j) = Sigmafj_mi_m(j) + Meanf(i - 1, j - 1)
    Next i
  Next j

  ' Now run the analysis again, using the mean data and the mean value of phi
  Phi = MeanPhi
  CalculateAlphas
  CalculateExpecteds

  ' And finally write out the Meanf(i, j) and their corresponding fHat(i, j)s
  For i = 1 To 60
    For j = 1 To 8
      Worksheets("Calculation Sheet").Cells(i + 1, (j * 2) + 12).Value = ""
      Worksheets("Calculation Sheet").Cells(i + 1, (j * 2) + 13).Value = ""
    Next j
  Next i
  For j = StartStage To EndStage
    For i = StartDay To EndDay
      Worksheets("Calculation Sheet").Cells(i + 1, (j * 2) + 12).Value = f(i, j)
    Next i
  Next j
  For j = StartStage + 1 To EndStage
    For i = StartDay + 1 To EndDay
      Worksheets("Calculation Sheet").Cells(i + 1, (j * 2) + 13).Value = fHat(i, j)
    Next i
  Next j
End Sub



' ***** FindBestSDPhi routines *****

Private Sub CalculateAlphas()

' Calculates the Transition rates of each stage to the next

  Dim j As Integer
  For j = EndStage To StartStage + 1 Step -1
    If Sigmafj_mi_m(j) > 0 Then Alpha(j - 1) = _
      ((Sigmafji(j) / Phi) - (Sigmafji_m(j) * (1 - Alpha(j)))) / Sigmafj_mi_m(j)
  Next j
  For j = StartStage To EndStage
    Worksheets("Calculation Sheet").Cells(j + 6, 6).Value = Alpha(j)
  Next j
End Sub



Private Sub CalculateExpecteds()

' Calculates the expected values, fHat(i, j) for each stage and time-step

  Dim j, i As Integer
  For i = StartDay To EndDay
    For j = StartStage To EndStage
      fHat(i, j) = 0
    Next j
```

```
  Next i
  For i = StartDay To EndDay
    fHat(i, StartStage) = f(i, StartStage)
  Next i
  For j = StartStage To EndStage
    fHat(StartDay, j) = f(StartDay, j)
  Next j
  For j = StartStage + 1 To EndStage
    For i = StartDay + 1 To EndDay
      fHat(i, j) = _
        ((fHat(i - 1, j - 1) * Alpha(j - 1)) + (fHat(i - 1, j) * (1 - Alpha(j)))) * Phi
    Next i
  Next j
End Sub


Private Sub CalculateSDPhi()

' Calculates the Standard Deviation of the deviations of the expected fHat(i, j)s from
' the observed f(i, j) values.

  Dim j, i, NCount As Integer
  Dim SigmaDN As Double
  SigmaDN = 0
  NCount = 0
  For i = StartDay + 1 To EndDay
    For j = StartStage + 1 To EndStage
      SigmaDN = SigmaDN + ((f(i, j) - fHat(i, j)) ^ 2)
      NCount = NCount + 1
    Next j
  Next i
  SDPhi = Sqr(SigmaDN / (NCount - 1))
  Worksheets("Calculation Sheet").Cells(4, 6).Value = SDPhi
End Sub
```

Appendix B

This is a listing of the Microsoft Excel 95 Visual Basic program used for the analysis described in Section 5.4.4

```
' This is an alternative method based on Manly, B.F.J. (1987) Res. Popul. Ecol. 29:119-127
' It includes stage-specific survival rates and uses multiple regression to estimate
' parameters.
'
' The macro was written by Douglas J. Reed in May 1998
'
' The procedures reference two other worksheets: the "Regression Sheet" holds the
' initial values to use for the parameters and displays the results. "Complete Counts"
' holds all of the stage-frequency counts for the analysis.

' Ensures that variable names are checked for errors, helping to trap bugs
Option Explicit

' RegressionRecord is used to pass all of the information required for the regression
' procedure in a convenient package.

Type RegressionRecord
       nX As Integer
       num As Integer
       x(7, 60) As Double
       y(60) As Double
       Coeff(7) As Double
End Type

' MeanValuesRecord is used for parameters that collect the data from all the replicates
' and calculate means

Type MeanValuesRecord
       N As Integer
       Replicate(10) As Double
End Type

' The global variables required by the procedures in the program. Their names mostly
' correspond to the parameters used in the model.

Dim Treatment, StartDay, EndDay, SampleNumber As Integer
Dim RepNumber, CurrentRep, StageNumber, Stages(7, 2) As Integer
Dim f(60, 7), G(60, 7), Phi(8) As Double
Dim BHat(60, 7), MHat(8), MuHat(8), aHat(8), OmegaHat(8) As Double
Dim RepaHats(8) As MeanValuesRecord
Dim RepOmegaHats(8) As MeanValuesRecord
Dim RepPhis(8) As MeanValuesRecord

Dim Data As RegressionRecord


Sub RegressionMainProc()

  ' This is the main procedure that is called by the Run button on the Regression Sheet
  ' It loops through the replicates, performing the analysis on each one in turn.
```

```
  Dim RepCount, a, b As Integer
  Dim p As Double
  GetSetupValues
  For RepCount = 1 To RepNumber
    CurrentRep = RepCount
    GetRepValues
    FindPhis
    CalculateBHats
    CalculateOtherParams
  Next RepCount
  CalculateMeans
End Sub


' ***** RegressionMainProc Procedures *****

Private Sub GetSetupValues()

' Gets the initial parameters for the run from the Regression Sheet

  Dim a, b As Integer

  Treatment = Worksheets("Regression Sheet").Range("B3").Value
  RepNumber = Worksheets("Regression Sheet").Range("B4").Value
  StartDay = Worksheets("Regression Sheet").Range("B5").Value
  EndDay = Worksheets("Regression Sheet").Range("B6").Value
  StageNumber = Worksheets("Regression Sheet").Range("B8").Value

  ' This section allows stages to be lumped together if necessary
  For a = 1 To StageNumber
    Stages(a, 1) = Worksheets("Regression Sheet").Cells(a + 10, 2).Value
    Stages(a, 2) = Worksheets("Regression Sheet").Cells(a + 10, 3).Value
  Next a

  For b = 1 To 8
    RepPhis(b).N = 0
    RepaHats(b).N = 0
    RepOmegaHats(b).N = 0
  Next b
End Sub


Private Sub GetRepValues()

' Gets the parameters for a specific replicate

  Dim CurrentRow, RowOffset, j, i, h, s As Integer
  Dim FoundData As Boolean
  Worksheets("Regression Sheet").Range("G2").Value = CurrentRep
  RowOffset = (((Treatment / 4) - 1) * 600) + ((CurrentRep - 1) * 60) + 1
  EndDay = Worksheets("Regression Sheet").Range("B6").Value
  StageNumber = Worksheets("Regression Sheet").Range("B8").Value

  'Get the counts for each stage and day and put them into f(i,j)
  For h = StartDay To EndDay
    i = h - StartDay + 1
    CurrentRow = h + RowOffset
    For j = 1 To StageNumber
      f(i, j) = 0
      For s = Stages(j, 1) To Stages(j, 2)
        f(i, j) = f(i, j) + Worksheets("Complete Counts").Cells(CurrentRow, s + 3).Value
```

```
      Next s
    Next j
  Next h
  SampleNumber = EndDay - StartDay + 1

  ' Find the last day to contain data
  i = SampleNumber + 1
  FoundData = False
  Do
    i = i - 1
    For j = 1 To StageNumber
      If f(i, j) > 0 Then FoundData = True
    Next j
  Loop Until FoundData
  SampleNumber = i
  Worksheets("Regression Sheet").Range("H2").Value = SampleNumber + StartDay - 1

  ' Find the last stage to contain data
  s = StageNumber + 1
  Do
    s = s - 1
    h = SampleNumber + 1
    Do
      h = h - 1
    Loop Until h = 0 Or f(h, s) > 0
  Loop Until h > 0
  StageNumber = s
  Worksheets("Regression Sheet").Range("I2").Value = StageNumber

  'Calculate the total counts, G(i,j)
  For i = 1 To SampleNumber
    For j = 1 To StageNumber
      G(i, j) = 0
      For s = j To StageNumber
        G(i, j) = G(i, j) + f(i, s)
      Next s
    Next j
  Next i

End Sub


Private Sub FindPhis()

  ' Narrows down the estimates of Phi for each stage by removing Phis >1

  Dim WholeData As RegressionRecord
  Dim a, b, c, s(8), t As Integer
  Dim eG As Double
  Dim Success As Boolean

  ' First save the complete dataset in WholeData
  WholeData.nX = StageNumber
  WholeData.num = SampleNumber - 1
  For a = 1 To WholeData.nX
    WholeData.Coeff(a) = 0
    For b = 1 To WholeData.num
      WholeData.x(a, b) = f(b, a)
    Next b
  Next a
  For b = 1 To WholeData.num
    WholeData.y(b) = G(b + 1, 1)
```

```
Next b

' Now display the values on the spreadsheet, just to check
For a = 1 To 60
  For b = 1 To WholeData.nX + 2
    Worksheets("Regression Sheet").Cells(a + 1, (b * 2) + 12).Value = ""
  Next b
Next a
For b = 1 To WholeData.nX
  For a = 1 To WholeData.num
    Worksheets("Regression Sheet").Cells(a + 1, (b * 2) + 12).Value = _
      WholeData.x(b, a)
  Next a
Next b
For a = 1 To WholeData.num
  Worksheets("Regression Sheet").Cells(a + 2, ((WholeData.nX + 1) * 2) + 12) _
    .Value = WholeData.y(a)
Next a

' Now transfer WholeData to Data
Data.nX = WholeData.nX
Data.num = WholeData.num
For b = 1 To Data.num
  Data.y(b) = WholeData.y(b)
  For a = 1 To Data.nX
    Data.x(a, b) = WholeData.x(a, b)
    Data.Coeff(a) = 0
  Next a
Next b

' Loop through, successively removing terms and reanalysing the data until
' none of the Phis are > 1
For a = 1 To Data.nX
  s(a) = a
Next a
Success = False
t = WholeData.nX
Regression
c = 1
Do
  If Data.Coeff(c) >= 1 Then
    ' Remove the data for j = c from the analysis ...
    WholeData.Coeff(s(c)) = 1
    Data.nX = Data.nX - 1

    '...subtract the data from y...
    For b = 1 To Data.num
      Data.y(b) = Data.y(b) - Data.x(c, b)
    Next b

    ' ...then shift all of the x values down to fill the gap.
    For a = c To Data.nX
      s(a) = s(a + 1)
      For b = 1 To Data.num
        Data.x(a, b) = Data.x(a + 1, b)
      Next b
    Next a
    Regression
  Else
    WholeData.Coeff(s(c)) = Data.Coeff(c)
    c = c + 1
  End If
Loop Until c > Data.nX
```

```
  For a = 1 To WholeData.nX
    Worksheets("Regression Sheet").Cells(a + 3, 6).Value = WholeData.Coeff(a)
  Next a
  For a = 1 To WholeData.nX
    Phi(a) = WholeData.Coeff(a)
    RepPhis(a).N = RepPhis(a).N + 1
    RepPhis(a).Replicate(RepPhis(a).N) = Phi(a)
  Next a

  ' creates estimated values of G(i,1) based on the model estimates
  For b = 1 To WholeData.num
    eG = 0
    For a = 1 To WholeData.nX
      eG = eG + (WholeData.Coeff(a) * WholeData.x(a, b))
    Next a
    Worksheets("Regression Sheet").Cells(b + 2, ((WholeData.nX + 2) * 2) + 12).Value = eG
  Next b
End Sub


Private Sub CalculateBHats()

  ' Calculates the estimated values of B(i,j), the number entering stage j and higher
  ' between times i and i+1

  Dim i, j, c As Integer
  For i = 1 To 60
    For j = 1 To 7
      Worksheets("Regression Sheet").Cells(i + 1, j + 32).Value = ""
    Next j
  Next i
  For j = 1 To StageNumber
    For i = 1 To SampleNumber - 1
      BHat(i, j) = G(i + 1, j)
      For c = j To StageNumber
        BHat(i, j) = BHat(i, j) - (Phi(c) * f(i, c))
      Next c
      Worksheets("Regression Sheet").Cells(i + 1, j + 32).Value = BHat(i, j)
    Next i
  Next j
End Sub


Private Sub CalculateOtherParams()

  ' Calculates other parameters, based on the values of BHat calculated above.
  ' MHat is the estimated total number entering each stage.
  ' MuHat is the estimated mean time of entry to each stage.
  ' aHat is the estimated mean stage duration.
  ' OmegaHat is the estimated through-stage survival.

  Dim j, i As Integer

  ' Calculate MHats
  For j = 1 To StageNumber
    MHat(j) = G(1, j)
    For i = 1 To SampleNumber - 1
      MHat(j) = MHat(j) + BHat(i, j)
    Next i
  Next j
```

```
  ' Calculate MuHats
  For j = 1 To StageNumber
    MuHat(j) = 0
    For i = 1 To SampleNumber - 1
      MuHat(j) = MuHat(j) + (i * BHat(i, j))
    Next i
    MuHat(j) = MuHat(j) / MHat(j)
  Next j

  'Calculate aHats
  For j = 1 To StageNumber - 1
    aHat(j) = MuHat(j + 1) - MuHat(j)
    Worksheets("Regression Sheet").Cells(j + 3, 8).Value = aHat(j)
    RepaHats(j).N = RepaHats(j).N + 1
    RepaHats(j).Replicate(RepaHats(j).N) = aHat(j)
  Next j

  'Calculate OmegaHats
  For j = 1 To StageNumber - 1
    OmegaHat(j) = MHat(j + 1) / MHat(j)
    Worksheets("Regression Sheet").Cells(j + 3, 9).Value = OmegaHat(j)
    RepOmegaHats(j).N = RepOmegaHats(j).N + 1
    RepOmegaHats(j).Replicate(RepOmegaHats(j).N) = OmegaHat(j)
  Next j
End Sub


Private Sub CalculateMeans()

  ' Calculates mean values and standard errors for the model estimates.

  Dim j, k As Integer
  Dim MeanSurvival(8), MeanDuration(8), MeanPhi(8) As Double
  Dim SEPhi(8), SESurvival(8), SEDuration(8) As Double
  Dim SSPhi(8), SSSurvival(8), SSDuration(8) As Double
  StageNumber = Worksheets("Regression Sheet").Range("B8").Value

  ' calculate mean stage-specific survival values
  For j = 1 To StageNumber
    MeanPhi(j) = 0
    SEPhi(j) = 0
    For k = 1 To RepPhis(j).N
      MeanPhi(j) = MeanPhi(j) + RepPhis(j).Replicate(k)
    Next k
    MeanPhi(j) = MeanPhi(j) / RepPhis(j).N
    Worksheets("Regression Sheet").Cells(12 + j, 6).Value = MeanPhi(j)
  Next j

  ' calculate standard error of the mean Phis
  For j = 1 To StageNumber
    SSPhi(j) = 0
    For k = 1 To RepPhis(j).N
      SSPhi(j) = SSPhi(j) + ((RepPhis(j).Replicate(k) - MeanPhi(j)) ^ 2)
    Next k
    SEPhi(j) = Sqr((SSPhi(j) / (RepPhis(j).N - 1)) / RepPhis(j).N)
    Worksheets("Regression Sheet").Cells(12 + j, 7).Value = SEPhi(j)
  Next j

  ' Calculate mean and standard error for through-stage survivals
  For j = 1 To StageNumber - 1
    MeanSurvival(j) = 0
    For k = 1 To RepOmegaHats(j).N
```

```
      MeanSurvival(j) = MeanSurvival(j) + RepOmegaHats(j).Replicate(k)
    Next k
    MeanSurvival(j) = MeanSurvival(j) / RepOmegaHats(j).N
    Worksheets("Regression Sheet").Cells(12 + j, 10).Value = MeanSurvival(j)
  Next j
  For j = 1 To StageNumber - 1
    SSSurvival(j) = 0
    For k = 1 To RepOmegaHats(j).N
      SSSurvival(j) = SSSurvival(j) + ((RepOmegaHats(j).Replicate(k) - MeanSurvival(j)) ^ 2)
    Next k
    SESurvival(j) = Sqr((SSSurvival(j) / (RepOmegaHats(j).N - 1)) / RepOmegaHats(j).N)
    Worksheets("Regression Sheet").Cells(12 + j, 11).Value = SESurvival(j)
  Next j

  ' Calculate mean and standard error for durations
  For j = 1 To StageNumber - 1
    MeanDuration(j) = 0
    For k = 1 To RepaHats(j).N
      MeanDuration(j) = MeanDuration(j) + RepaHats(j).Replicate(k)
    Next k
    MeanDuration(j) = MeanDuration(j) / RepaHats(j).N
    Worksheets("Regression Sheet").Cells(12 + j, 8).Value = MeanDuration(j)
  Next j
  For j = 1 To StageNumber - 1
    SSDuration(j) = 0
    For k = 1 To RepaHats(j).N
      SSDuration(j) = SSDuration(j) + ((RepaHats(j).Replicate(k) - MeanDuration(j)) ^ 2)
    Next k
    SEDuration(j) = Sqr((SSDuration(j) / (RepaHats(j).N - 1)) / RepaHats(j).N)
    Worksheets("Regression Sheet").Cells(12 + j, 9).Value = SEDuration(j)
  Next j
End Sub


' ***** FindPhis procedures *****

Private Sub Regression()

  ' This procedure is called by FindPhis and performs a multiple regression analysis on
  ' the data given to it in Data.

  Dim a, b, c As Integer
  Dim Meanx(100), Meany, Matrix(10, 11), TempMatrix(1, 11), SumOfProducts, z As Double

  ' calculate mean values of each x and for y.
  For a = 1 To Data.nX
    Meanx(a) = 0
    For b = 1 To Data.num
      Meanx(a) = Meanx(a) + Data.x(a, b)
    Next b
    Meanx(a) = Meanx(a) / Data.num
  Next a
  Meany = 0
  For b = 1 To Data.num
    Meany = Meany + Data.y(b)
  Next b
  Meany = Meany / Data.num

  ' Now calculate the sums of squares and put them in the matrix
  For a = 1 To Data.nX
    For b = 1 To Data.nX
      SumOfProducts = 0
```

```
      For c = 1 To Data.num
        SumOfProducts = SumOfProducts + (Data.x(a, c) * Data.x(b, c))
      Next c
      Matrix(a, b) = SumOfProducts
    Next b
  Next a
  For a = 1 To Data.nX
    SumOfProducts = 0
    For c = 1 To Data.num
      SumOfProducts = SumOfProducts + (Data.x(a, c) * Data.y(c))
    Next c
    Matrix(a, Data.nX + 1) = SumOfProducts
  Next a

  ' Use gaussian elimination on the matrix
  For c = 2 To Data.nX
    ' sort the matrix by column c-1
    For a = c To Data.nX
      If Matrix(a, c - 1) > Matrix(c - 1, c - 1) Then
        For b = 1 To Data.nX + 1
          TempMatrix(1, b) = Matrix(c - 1, b)
          Matrix(c - 1, b) = Matrix(a, b)
          Matrix(a, b) = TempMatrix(1, b)
        Next b
      End If
    Next a
    For a = c To Data.nX
      z = Matrix(a, c - 1) / Matrix(c - 1, c - 1)
      For b = c - 1 To Data.nX + 1
        Matrix(a, b) = Matrix(a, b) - (Matrix(c - 1, b) * z)
      Next b
    Next a
  Next c
  Data.Coeff(Data.nX) = Matrix(Data.nX, Data.nX + 1) / Matrix(Data.nX, Data.nX)

  ' calculate the coefficients
  For a = Data.nX - 1 To 1 Step -1
    z = Matrix(a, Data.nX + 1)
    For b = Data.nX To a + 1 Step -1
      z = z - (Data.Coeff(b) * Matrix(a, b))
    Next b
    Data.Coeff(a) = z / Matrix(a, a)
  Next a
End Sub
```

Appendix C: *Oecologia* 1996 paper

This is the text of a paper published in 1996 in *Oecologia* **105**:189-193.

Differential cannibalism and population dynamics in a host-parasitoid system

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Abstract

The effects of host cannibalism on a host-parasitoid system were explored through experiment and modelling. In individual encounters between parasitized and unparasitized *Plodia interpunctella* larvae, parasitized larvae were more likely to be cannibalized. Inclusion of this differential cannibalism into a simple Lotka-Volterra type model of host-parasitoid population dynamics generates alternative stable states -- including stable coexistence and extinction of the parasitoid -- which depend on starting conditions. Possible mechanisms for differential cannibalism, and its implications for studies of host-parasitoid populations and biological control programmes are discussed.

Keywords: *Plodia interpunctella*, *Venturia canescens*, cannibalism, population dynamics, biological control.

Introduction

Cannibalism is a common phenomenon in many animal species (Fox, 1975; Polis, 1981), often accounting for very high levels of mortality in a population. The effects of cannibalism on single-species systems have been explored extensively in models of population dynamics (Polis, 1981). However, little attention has been paid to the role of cannibalism in the interactions of two or more species, such as those between a pathogen or parasite and its cannibalistic host.

Cannibalism can be highly advantageous to the cannibal, since such a highly nutritious diet often leads to increased survival and reproductive success (Joyner and Gould, 1985). However, cannibalism can also be costly, leading to the risk of injury from the victims' defence, and reducing inclusive fitness if closely related individuals are cannibalized (Pfennig et al., 1993). When diseases or parasites can be acquired through eating infected conspecifics, this can add a strong extra risk to cannibalism (Polis, 1981; Elgar and Crespi, 1992). Transmission of pathogens and parasites by cannibalism has been shown in several species (Schaub, 1988; Matuschka and Bannert, 1989; Schaub et al., 1989) and may significantly increase the risk of infection and death (Pfennig et al., 1991). However, Hart (1990) suggests that where diseases or parasites are not transmitted by ingestion of infected tissue, cannibalism may *prevent* the spread of infection by removing infectious individuals from the population. Parasitoids can be useful study animals to test this last theory, since their insect hosts can show high levels of cannibalism, and the parasitoid larvae are killed as the host is eaten, so there is no chance of cross-infection. If parasitized hosts are more often cannibalized than unparasitized ones, there could be significant effects on the population dynamics both of the host and of the parasitoid. Here we explore the effects of cannibalism on a well studied host-parasitoid system, both by experiment, to see whether parasitized hosts suffer greater levels of cannibalism, and through a model which examines the potential effects on the population dynamics of the system.

Methods

The hosts were *Plodia interpunctella* (Hübner) larvae, which were cultured on a 10:1:1 mixture of wheat bran, yeast and glycerol. The parasitoid was *Venturia canescens* (Gravenhorst), which readily parasitizes the later instars of *P. interpunctella*. Stock cultures were kept at 25 C in a controlled temperature room with a 16:8 hour light:dark cycle. The experiment was conducted under the same conditions, in 1.5ml tapered centrifuge tubes with small air holes punched in the lids.

For the experiment, early fourth instar host larvae were taken from culture and individually parasitized by placing each one under a glass vial with a single *V. canescens*, and waiting until the wasp laid an egg in it. *V. canescens* uses a characteristic 'cocking' motion of its ovipositor after laying an egg (Rogers, 1972), so it was possible to guarantee that each larva had been parasitized. The parasitized larvae were then placed into

individual tubes half filled with food. Equal numbers of unparasitized larvae were also set up in individual tubes after being handled in the same way as the parasitized larvae. The larvae were left for four days to develop to early fifth instar stage, by which time the parasitoid larvae should have hatched and begun feeding on the host (Salt, 1968). They were then randomly assigned to new, empty tubes as one of three treatments: 1 -- a parasitized and an unparasitized larva together, to test for cannibalism; 2 -- a parasitized larva alone, to determine the rate of parasitoid encapsulation; 3 -- an unparasitized larva alone, to monitor the mortality rate of larvae during the experiment. The larvae were left for 48 hours for cannibalism to take place in treatment 1, before the number of larvae remaining alive in each tube was counted and food was added. The larvae were reared through and the number of adult wasps and moths emerging in each tube was counted.

Results

Cannibalism was assumed to have occurred in treatment 1 (parasitized with unparasitized larvae) if only one larva remained after 48 hours. The results are summarized in Table 1. One hundred and twenty-five of the 133 replicates of treatment 1 were successfully reared through to produce adult moths and/or wasps, and cannibalism occurred in 59 of these. In treatment 2 (parasitized larva on its own), 7 out of 115 hosts developed into moths, and can be assumed to have encapsulated the developing parasitoid, giving an encapsulation rate of 0.061. The null hypothesis for the experiment was that parasitism would have no effect on cannibalism, so that equal numbers of parasitized and unparasitized larvae would survive. After adjusting for the encapsulation rate of parasitoids, this gives expected values of 31.3 moths and 27.7 parasitoids for treatment 1. These expected values are significantly different from the observed numbers emerging ($X^2 = 5.15$, d.f. = 1, $p<0.03$). Mortality rates of parasitized (treatment 2) and unparasitized (treatment 3) larvae were very similar ($X^2 = 0.07$, d.f. = 1, $p>0.79$) hence there is no evidence to support the idea that differences in emergence in treatment 1 were due to differential mortality.

Model

The experiment has shown that *P. interpunctella* larvae that are parasitized by *V. canescens* suffer a higher risk of being cannibalized than unparasitized larvae. We now investigate the possible effects of this on the population dynamics of host-parasitoid interactions by examining the following simple model based on predator-prey dynamics:

$$\frac{dH}{dt} = rH - qH^2 - aHP - c_H H^3 \ , \qquad (1)$$

$$\frac{dP}{dt} = faHP - mP - c_P H^2 P \ . \qquad (2)$$

The density-dependent terms, especially in the prey equation (1), are designed to overcome one of the major shortcomings of the original Lotka-Volterra formulation, on which the model is based. The prey or host population (density *H*), with an intrinsic rate of increase *r*, is subject to self-regulation (coefficient of crowding, *q*), to predation by a predator or parasitoid (population density *P*, attack rate *a*), but also to cannibalism. This occurs at a per capita rate $c_H H$, reflecting the usual finding that the rate of cannibalism is density-dependent (Polis, 1981). When combined with an encounter rate between 'cannibal' hosts and 'victim' hosts, proportional to H^2, this gives rise to the term $-c_H H^3$ in equation (1). Turning to equation (2), the parasitoid increases in density by converting consumed hosts into parasitoid offspring (conversion efficiency *f*). It is subject to natural mortality (at rate *m*) but also, crucially here, to extra predation (as parasitized hosts) by healthy hosts (differential cannibalism). This occurs at a per capita rate $c_H H$, again density-dependent, and when combined with an encounter rate between healthy hosts and parasitized hosts proportional to *PH*, this gives rise to the term $-c_H H^2 P$ in equation (2).

Note that for both hosts and parasitoids, the equations subsume adults and larvae into single variables, as is the case in the vast majority of predator-prey and parasitoid-host models. The added reality of density-dependence makes this less easy to justify (since adults reproduce but crowding amongst larvae may be the main constraint), and this is especially the case with cannibalism, which explicitly affects the larvae of both hosts and parasitoids. Nonetheless, the present model, in introducing the notion of cannibalism in two-species systems for the first time, and in a tractable form, can be expected to capture the essence of its effects on the dynamics of the populations.

The behaviour of this model may be investigated most simply and transparently by deriving zero isoclines for both parasitoid and host and examining the behaviour of joint populations graphically. These are:

host zero isocline: $P = -\frac{c_H}{a}H^2 - \frac{q}{a}H + \frac{r}{a}$,

parasitoid zero isocline: $H = \frac{fa \pm i}{2c}$,

where $i = \sqrt{f^2a^2 - 4cm}$.

Simple algebraic manipulation shows the host zero isocline to be a simple curve in the quadrant where both host and parasitoid densities are positive (Fig. 1a). Host density increases when both parasitoid and host densities are relatively low, but decreases when either or both are relatively high, reflecting the effects on host density of both parasitism and self-regulation. When parasitoid density is zero, host density stabilizes at a 'carrying capacity' given by:

$$H = \frac{\left(-q + \sqrt{q^2 + 4c_H r}\right)}{2c_H} .$$

Thus, as expected, the carrying capacity increases with increasing *r* but decreases with increasing *q* and c_H.

The parasitoid zero isoclines are less straightforward. Note first that their existence in a biologically-relevant form requires $i \geq 0$. In other words, unsurprisingly, the parameters defining the mortality effects on the parasitoid (c_P, *m*) must not be too great relative to those defining the parasitoid's intrinsic rate of increase (*f*, *a*). With this proviso, there are two isoclines (Fig. 1b) both of which run parallel to the parasitoid axis (*H* constant). These combine for $i = 0$. Below the isocline at the lower host density, parasitoid density decreases as a result of the lack of hosts. Above the isocline at the higher host density, parasitoid density again decreases -- this time as a result of host cannibalism. Parasitoid density increases between the two isoclines.

There are three ways in which these isoclines can be juxtaposed. If both parasitoid isoclines lie beyond the intercept of the host isocline on the host axis (Fig. 1c), then the only stable outcome has the parasitoid absent and the host population at its carrying

capacity. There are not sufficient hosts to sustain a population of parasitoids even at the host's carrying capacity.

If only the lower parasitoid isocline intercepts the host isocline (Fig. 1d), then the only stable outcome is a joint parasitoid-host equilibrium, which is approached through a series of damped, coupled oscillations in the two populations (Rosenzweig and MacArthur, 1963). The hosts are unable to depress the parasitoid population through differential cannibalism even at their own carrying capacity.

However, if both parasitoid isoclines intercept the host isocline (Fig. 1e), then there are alternative stable states, with the exact outcome contingent on initial densities or densities arrived at through chance or externally-driven fluctuations. The system may be attracted to a joint parasitoid-host equilibrium, since f and a for the parasitoid, and r for the host, are sufficiently large, and q sufficiently small, for the host to be able, at and below their own carrying capacity, to sustain a population of parasitoids. But the system may also be attracted to an equilibrium with the host at its carrying capacity and the parasitoid absent, since parasitoid mortality (m) is sufficiently large and levels of cannibalism (c_H and c_P) are great enough to depress parasitoid density below a viable level.

The positions of the parasitoid zero isoclines are very sensitive to the level of cannibalism of parasitized larvae (c_P). As c_P increases, the isoclines converge rapidly, with the higher isocline moving more quickly towards lower host densities. The host zero isocline, and in particular the carrying capacity where $P = 0$, is much less affected by changes in c_H. Thus, the situation in Fig. 1e can be acheived either through generally high levels of cannibalism (c_H and c_P both large), or by differential cannibalism of parasitized hosts ($c_P > c_H$).

Hence, this model makes it clear that in host-parasitoid interactions such as those described here, where cannibalistic hosts show a preference for parasitized hosts, the potential exists for irregular fluctuations in abundance, as the system moves, perhaps as a result of chance fluctuations, from one zone of attraction to another. Similarly, the results of introducing parasitoids into a population of hosts may be variable, and to some degree unpredictable, depending on precisely how many parasitoids are introduced and the precise density of hosts at the time. This model could also be potentially used to describe any host-density dependent effect that increases the mortality of parasitized hosts, such

as starvation, and is not limited to the case of differential cannibalism that we discuss here.

Discussion

This appears to be the first study to have explored the idea that parasitoid population dynamics could be affected by host cannibalism. The experiment has shown that cannibalism can lead to differential mortality of parasitized and unparasitized hosts, while modelling of host-parasitoid population dynamics has revealed that this differential cannibalism can generate alternative stable states and even extinction of the parasitoid.

In order to encourage cannibalism to take place, no food was made available to the *P. interpunctella* larvae during the experiment. It has been suggested that cannibalism is often a laboratory artefact, caused by extreme conditions of starvation or density (Fox, 1975), however some studies have shown that cannibalism will occur even in the presence of abundant food and low density (Dial and Adler, 1990; Van den Bosch and Santer, 1993). Our own observations suggest that this is also the case for *P. interpunctella*.

The experimental results do not show how differential cannibalism comes about. Potential mechanisms would probably involve either a change in the behaviour of parasitized larvae, making them more vulnerable to cannibalism, or detection and preferential attack of parasitized larvae by cannibals. An immature parasitoid can have profound effects on the behaviour and development of its host (Godfray, 1994, ch. 6), and this could affect the vulnerability of the host to cannibalism. *V. canescens* is known to coat it's eggs in virus-like particles which weaken the host's immune system and prevent encapsulation (Rotheram, 1967). Once the parasitoid larva hatches it rapidly feeds on the host's haemolymph, which depresses its immune system even further, and forces the host to use up fat reserves to compensate (Salt, 1968). These effects will probably combine to weaken the host larva, making it less able to resist attack by cannibals, and less likely to attack other larvae. The parasitoid may also slow down the host's development by depleting resources, making it smaller than an unparasitized larva of the same age (Harvey et al., 1994). Smaller individuals are usually more susceptible to cannibalism, but this situation can be reversed if the larger individual still has a soft cuticle after moulting, and so is more vulnerable to attack (Dial and Adler, 1990). Finally, cannibals may preferentially attack parasitized individuals. Such a mechanism would involve detection of the

parasitoid larva within a parasitized host by the cannibal, possibly using chemical or behavioural cues.

Whichever mechanism is responsible for differential cannibalism, the end result is that the number of parasitized individuals in a population of hosts will be reduced compared to a population in which no differential cannibalism occurs. This, in turn, will lead to fewer parasitoids present in the next generation to parasitize hosts. Thus, our findings support Hart's theory (1990) that cannibalism could, in some cases, control the spread of parasitism. The costs of differential cannibalism are more difficult to determine, however, since some of the normal costs of cannibalism do not apply in this case. For instance, once a *P. interpunctella* larva has been parasitized it will almost certainly die -- fewer than 7% of the parasitized larvae in this experiment were able to encapsulate the parasitoid and develop normally -- so even if the cannibal is closely related to its victim it does not suffer an appreciable loss in inclusive fitness through cannibalism. Also, the cannibal is probably less likely to be injured attacking a weakened, parasitized victim, so the main potential cost for the cannibal will not be injury, but rather the risk of becoming infected with other forms of disease or parasite which can be transmitted during cannibalism.

Our modelling of differential cannibalism in population dynamics has shown that it can produce widely varying effects that are strongly dependent on starting conditions. At some densities of host and parasitoid, differential cannibalism will still allow convergence towards a stable coexistence, while at other densities the parasitoid may die out altogether. The model is not limited to the case of differential cannibalism and could be applied to any situation where mortality of parasitized hosts is host-density dependent. A similar model, but using a Nicholson-Bailey formulation, was developed by Bernstein (1986) who added terms for the severity of density dependence and for the difference in susceptibility to competition of parasitized and unparasitized hosts to a model by May et al (1981). Bernstein's model shows, as we have done here, that as density dependent mortality of parasitized hosts increases then the possibility of extinction of the parasitoid becomes greater. However, his analysis did not consider the significance of starting conditions.

Starting conditions could have important implications for biological control programs where the right number of parasitoids must be introduced to control established host populations. If too few of the parasitoids are introduced, then they will not become

established, since the parasitized hosts will be cannibalized by healthy hosts before they are able to develop into new adult parasitoids. In a recent review of parasitoid releases for biological control (Hawkins et al., 1993), it was found that a large number of them failed to regulate the host species adequately. Differential cannibalism might provide a mechanism that acts in addition to the host refuges from parasitism that Hawkins et al. suggest to explain these failures.

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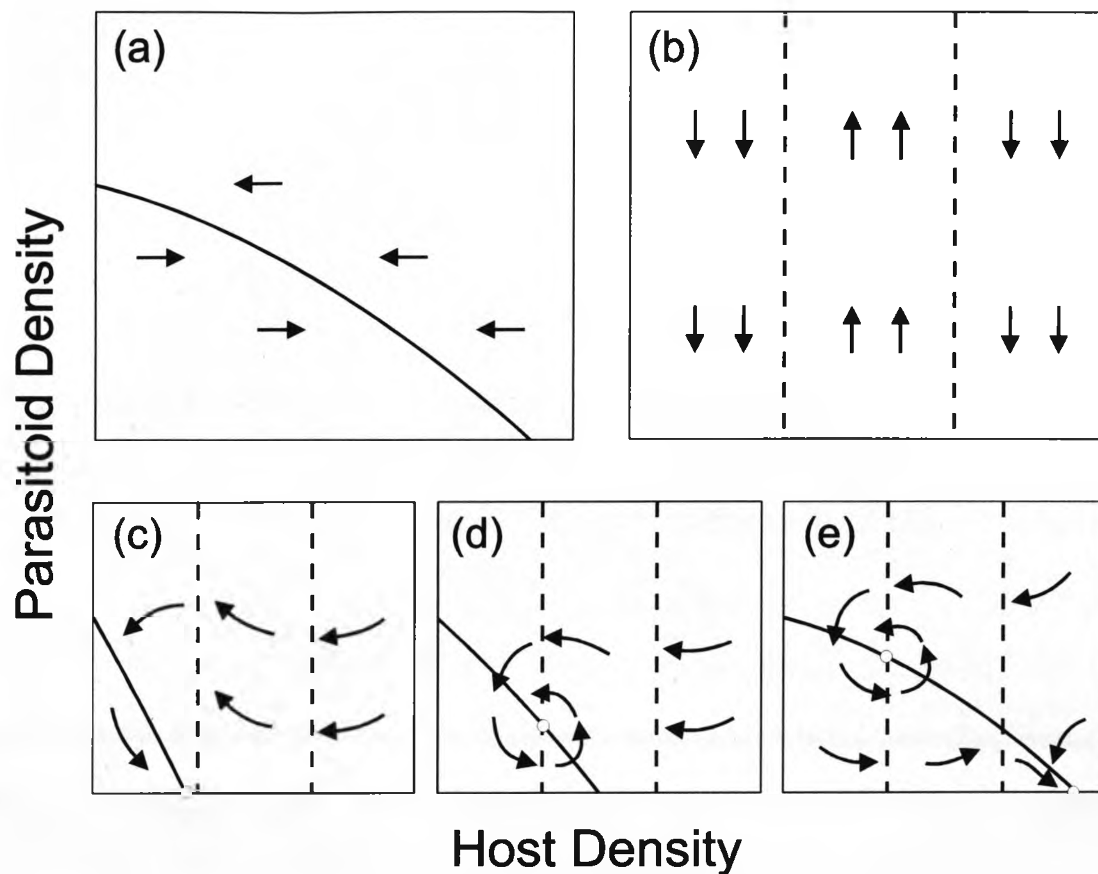

Fig. 1 **(a)** The host zero isocline for the model in equations (1) and (2), with zones of host increase and decrease indicated (arrows). **(b)** The parasitoid zero isoclines, with zones of parasitoid increase and decrease indicated. **(c)**, **(d)**, **(e)** The various possible juxtapositions of the isoclines. **(c)** 'Inefficient parasitoid': the system settles (O) at the host's carrying capacity (parasitoid eliminated). **(d)** 'Efficient parasitoid, inefficient differential cannibalism by the host': the system approaches (via damped cycles) and eventually settles at a joint parasitoid-host equilibrium. **(e)** 'Efficient parasitoid, efficient differential cannibalism by the host': the outcomes in **(c)** and **(d)** are alternative stable states.

		Treatment		
		1	2	3
Initial numbers of larvae	Parasitized	133	133	—
	Unparasitized	133	—	133
Nos. emerging after cannibalism	Parasitoids	19	—	—
	Moths	40	—	—
Nos. emerging after no cannibalism	Parasitoids	48	108	—
	Moths	64	7	117
Deaths due to cannibalism	Parasitoids	40	—	—
	Moths	19	—	—
Non-cannibalism deaths		36	18	16

Table 1. Initial numbers of hosts, parasitoid and moth emergence with and without cannibalism, and cannibalism and non-cannibalism mortality for the three experimental treatments (1=parasitized and unparasitized host together; 2=parasitized alone; 3=unparasitized alone).

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