# REPRODUCTIVE BEHAVIOUR OF *CNEPHASIA JACTATANA* WALKER (LEPIDOPTERA: TORTRICIDAE)

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2003

*Any good idea can be stated*

*in fifty words or less*

Stan Ulam

(1909-1984)

## Abstract

*Cnephasia jactatana* Walker is a pest of kiwifruit. Before this research, no information was available on its reproductive behaviour. Insects obtained from a mass-rearing system (MR) had a lower pupal weight, emergence rate and reproductive fitness than those from an individual-rearing (IR) system. Most moths emerged during the day with males emerging 1-2 d earlier than females. Females emerged with no mature eggs in their ovaries and required a 2-3 d egg maturation period before accepting mating. More than 50% of eggs were laid within 3 d after mating. Male fecundate capacity increased linearly with bodyweight whilst female fecundity had an upper bodyweight threshold. Mating delay affected females more than males in reproductive fitness. Delaying mating for 4 d reduced female reproductive fitness by > 85%. Female remating increased fecundity and fertility with MR females benefiting more than IR females from remating. Heavier females were more likely to remate than light females. Neither copula length nor spermatophore size influenced female remating behaviour. Few males mated twice within 24 h but > 50% males achieved 4 matings during their lifetime if they were allowed a recovery period of 24 h between matings. Multiple matings reduced male fertility and spermatophore size. Male mating history did not affect courtship and copula length. However, a longer activation time required by mated males reduced their chances of achieving a new mating. Males actively approached and courted females before mating occurred, while females appeared to be less active. Males used their antennae, labial palps and wings to touch females when courting. Virgin males performed more mating attempts than mated males when competing for females. Sexual selection in *C. jactatana* did not support Darwin's sexual selection theory where females are more selective and males suffer the sexual selection effects. In this species, males appeared to be more selective than females. Both sexes preferred virgin mates to mated mates for mating. Males preferred young to old females for mating while females did not discriminate the male age when choosing a mate. Male body weight did not confer any mating advantage. However, females preferred males with longer antennae for mating. Light- and average-weight males presented a size assortive-selection while heavy males did not show any preference.

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# CHAPTER 1
# GENERAL INTRODUCTION

## 1.1 Introduction

Leafrollers (Lepidoptera: Tortricidae) pose an economic threat to the fruit industry of New Zealand. They are important quarantine pests for kiwifruit (*Actinidia deliciosa*), apples (*Malus* spp.), avocado (*Persea americana*), blackcurrant (*Ribes nigrum*), blueberry (*Vaccinium corymbosum*), boysenberry (*Rubus loganobaccus*), *Citrus* spp., feijoa (*Feijoa sellowiana*), grape (*Vitis vinifera*), persimmon (*Diospyros kaki*) and raspberry (*Rubus idaeus*) among others (Steven 1990, van der Geest et al.1991).

Kiwifruit is an economically important crop for many countries in the world. For New Zealand, kiwifruit exports represent a return of NZ$ 476.2 million per year (Zespri 2002). Together Italy and New Zealand produced almost 50% of the world kiwifruit in 2002, while China and Chile accounted for 18%. Some minor producers are France, Iran, Greece, Japan, United States of America, and Spain (Kerr et al. 2001).

On unsprayed kiwifruit crops, damage caused by leafrollers can be as high as 40-50% (Steven 1990). *Cnephasia jactatana* Walker, known as the black-lyre leafroller, is an important component of the leafroller complex that attacks kiwifruit, grapes and raspberry (Steven 1990, van der Geest et al. 1991).

The methods used for the control of tortricids in New Zealand are dictated by export market requirements for pest-free produce. Because tortricids affecting kiwifruit are native to New Zealand, or are confined to Australasia, export markets outside the region require not only that fruit exports are free from insect damage but also that there is no risk of the fruit carrying live leafroller larvae (Wearing et al. 1991). Leafroller control in the late 1980's and early 1990's in New Zealand relied on the use of broad-spectrum organophosphates and carbamates (Steven 1990, van der Geest et al. 1991), generating insecticide resistance (Suckling et al. 1990, Caprio

and Suckling 1995, Lo et al. 2000) and residues on fruit (Blank et al. 1995). The presence of an unauthorised pesticide residue, or an authorised pesticide residue over the acceptable level, can result in the rejection of the consignment and lead to the loss of trade (Suckling et al. 2003).

To address this problem Steven et al. (1994) developed a first-stage integrated pest management system for kiwifruit, called "Kiwigreen", which reduced the use of pesticides. Current research aims to implement environmentally friendly control techniques such as the application of *Bacillus thuringensis* and sex pheromones (Steven et al. 1997). Although most leafrollers on kiwifruit are controlled by the current pest management programs, *C. jactatana* larvae, which burrow into the fruit, are on occasions still found at harvest (Stevens and McKenna 1999).

The increasing number of growers adopting an organic production system, the introduction of high-priced kiwifruit varieties, and the high phytosanitary standards demanded by the export market call for more research towards developing and improving current methods of pest control. Since most of the leafrollers associated with kiwifruit are native and restricted to New Zealand, information about the biology and ecology of these species is of crucial importance to the industry. The challenges and constraints of organic production, the emergence of new pests, and the potential susceptibility of new kiwifruit varieties to the established leafrollers demand in-depth studies of the biology of those species that comprise the kiwifruit leafroller pest complex.

## 1.2 Leafrollers on Kiwifruit

Most knowledge of the biology of economically important leafrollers in New Zealand is related to the brownheaded leafroller, *Ctenopseustis obliquana*, the light-brown apple moth, *Epiphyas postvittana*, and the greenheaded leafrollers complex, *Planotortrix excessana* and *Ctenopseustis obliquana*. This knowledge was gathered during the late 1980's and early 1990's when many studies of these species were carried out on apple and kiwifruit crops. However, in a comprehensive review of kiwifruit insect pests, by Steven (1990) and subsequent papers by Tomkins et al. (1991), Steven (1992), Stevens et al. (1993), Steven et al. (1994), and Stevens et al.

(1995), only a few lines are dedicated to the biology and ecology of other leafrollers. There is a paucity of knowledge of the biology and behaviour of *C. jactatana* contained in the literature.

According to Steven (1992), seven species of leafrollers are found in commercial kiwifruit orchards (Table 1.1). Except for *E. postvittana*, which was introduced to New Zealand from Australia in the 19th century, all leafroller species are native and restricted to New Zealand, representing a quarantine risk to countries which import New Zealand fruit (Steven 1990, van der Geest et al. 1991).

**Table 1.1** Leafrollers commonly found on kiwifruit in New Zealand[a]

| Scientific name | Plant part attacked | Local name |
|---|---|---|
| *Cnephasia jactatana* | Fruit, leaves | Black-lyre leafroller |
| *Ctenopseustis obliquana* | Fruit, leaves | Brownheaded leafroller |
| *Ctenopseustis herana* | Fruit, leaves | Brownheaded leafroller |
| *Epiphyas postvittana* | Fruit, leaves | Light brown apple moth |
| *Planotortrix excessana* | Fruit, leaves | Greenheaded leafroller |
| *Planotortrix octo* | Leaves | Greenheaded leafroller |
| *Planotortrix notophaea* | Leaves | White-striped leafroller |

[a] From Steven 1992.

The species *C. jactatana*, *Ct. obliquana*, and *Ct. herana* are considered the primary pests of kiwifruit whilst the importance of the other species is considered minor or uncertain (Steven 1992).

## 1.3 Importance of the Study of Reproductive Behaviour

The study of the reproductive behaviour of an insect helps us understand the life history and evolution of the species and enables us to predict the gene flow between populations (Cloutier et al. 2000). It can also provide information useful for the design and implementation of innovative monitoring or control tactics. Identification of a sex pheromone sets the foundation for implementing monitoring

or control techniques like mating disruption and trap and kill. Sex pheromones have been successfully used in New Zealand to monitor and manage pesticide-resistant populations of *Epiphyas postvittana* (Suckling et al. 1990, Caprio and Suckling 1995, Lo et al. 2000) and the development of an attracticide for controlling *E. postvittana* is underway (Brockerhoff and Suckling 1999). Similarly, the release of a large number of sterile insects into the field diminishes the number of fertile matings in the wild reducing the population pest. Study of the mating behaviour of the codling moth, *Cydia pomonella*, is mandatory for the success of its control through the Sterile Insect Technique (SIT); codling moth is under SIT control in Canada and USA (Bloem et al. 1998, Calkins 1998, Cossentine and Jensen 2000) and its implementation in South Africa was proposed by Riedl et al. in 1998. Other tortricids under investigation for the establishment of SIT programmes include *Grapholita molesta*, *Adoxophyes orana*, *Choristoneura fumiferana*, and *Zeiraphera diniana* (Butt 1991, Genchev and Gencheva 1995).

The study of reproductive behaviour is one of the most diverse and active fields in the biological sciences. For example, most lepidopteran females mate more than once regardless of the fact that most males deliver enough sperm in one mating to fertilise all the females' eggs, making female remating redundant (Drummond 1984). This apparent contradiction has provoked the development of many theories to explain the mating and remating behaviour and its consequences for the evolution of the species. Concepts like mate selection, sexual conflict and sperm competition among others, are the core of the terminology used in this field.

## 1.4 Relevance of this Research

To achieve economical production of high quality kiwifruit, good pest management of leafrollers is required. The lack of information on the biology and behaviour of *C. jactatana*, such as its reproductive behaviour, makes the development of its control methods difficult. Furthermore, such knowledge will facilitate a better understanding of the life story of the insect.

The study of *C. jactatana* is relevant because:

a) *Cnephasia jactatana* is native and endemic to New Zealand. The introduction of potential pests via trade has raised biosecurity concerns all over the world. *C.*

*jactatana* is considered a quarantine pest and New Zealand exporters must ensure that shipments are free of this pest. Being native and endemic to New Zealand means that only research done in the country will provide the necessary information to satisfy biosecurity standards.

b) *Cnephasia jactatana* is a widely distributed species in both main islands of New Zealand (Gaskin 1966). Apart from damaging kiwifruit, it has been collected from economically important plants such as apple, plum, gum, persimmon, pine, flax, grapes, and raspberry (Gaskin 1966, Spiller and Wise 1982, Green 1984, Steven 1990, van der Geest et al. 1991, Crowe 2002). It represents a potential risk for the commercial production of these commodities.

c) Little is known about its biology and its taxonomic position is not clear. The genus *Cnephasia* is represented by more than 25 species in New Zealand (Dugdale 1988) and only *C. jactatana* attacks fruit. In other parts of the world species of the genus *Cnephasia* are pests of cereals (Glas 1991). Minor references to *C. jactatana* are found in research papers dealing with leafrollers and most of what is known about its behaviour in the wild comes from anecdotal information. The pheromonal blend identified for *C. jactatana* (Foster et al. 1993) is different from that indicated by Roelofs and Brown (1982) as an attractant for the genus *Cnephasia*. Studies of the biology of this species may provide information useful for clarifying its taxonomic position.

d) The application of environmentally friendly techniques reduces the amount of pesticide used, thus facilitating access to new markets and increasing the value of the harvested produce. The study of *C. jactatana* reproductive behaviour provides information useful for the development of environmentally friendly IPM practices and understanding of the life story of the species. Unravelling the factors which govern the reproductive behaviour and its impact upon the reproductive fitness of the species, will provide valuable information for theoretical biologists and IPM developers.

In a very competitive international market, where retailers demand safe–to-eat fruit, and where other kiwifruit producing countries attempt to use pesticide residues as trade barriers for New Zealand kiwifruit (Blank et al. 1995), effective

IPM programs will ensure the acceptance and prevalence of New Zealand kiwifruit as a desirable commodity in the global market. This research intends to help control developers by providing lacking information of the biology of *C. jactatana.*

## 1.5 Aim and Objectives of This Study

The aim of this research is to provide useful information on the reproductive biology and mating behaviour of *C. jactatana* with the objectives as follows:

a) To investigate aspects of the basic biology and the population growth of *C. jactatana*

b) To determine the effect of mating delay and remating on reproductive fitness in *C. jactatana*

c) To investigate the mating behaviour and sexual selection of *C. jactatana*

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Introduction

This chapter reviews the current knowledge on the family Tortricidae relevant to my current studies. Special references are given to known biology of *Cnephasia jactatana* and reproductive behaviour of tortricids moths. When appropriate or necessary I include examples outside Tortricidae. Due to the topic and my personal interest, this revision is selective instead of all-inclusive.

## 2.2 Classification of *Cnephasia jactatana* Walker

In 1883, Walker described the tortricid *C. jactatana* from an insect collected in Nelson, New Zealand. The type specimen is deposited at the Natural History Museum in London. *Cnephasia jactatana* is the only species within the genus *Cnephasia* of any economic importance out of 28 reported by Dugdale (1988). According to Dugdale (1988), the proposed classification for this species is:

Family: Tortricidae

Subfamily: Tortricinae

Tribe: Archipini

Genus: *Cnephasia*

Species: *jactatana*

The validity of the classification for *C. jactatana* is questionable due to several factors: a) some morphological characters do not fit the *Cnephasia* genus (uncus not covered in setulae; the signum complete and daggered; $M_2$ muscle split and aedeagus unusually positioned in copulation) (Rawoski 1965), b) the functional component of *C. jactatana* female sex pheromone is (Z) - 11- tetradecenyl acetate (Z11-14:OAc) (Foster et al. 1993) instead of the expected Z9-12:OAc (Roelofs and

Brown 1982), which is characteristic of the Palearctic genus *Cnephasia* [e.g. *C. asseclana*, *C. longana* and *C. pumicana* (Arn 1991)] and c) members of the genus *Cnephasia* are cereal pests [e.g. *C. longana*, *C. pasivana* ] in Europe and USA (Glas 1991) while *C. jactatana* is a fruit pest.

## 2.3 Moth Description

The adult is a small moth with a wingspan of approximately 2 cm. The fore wings are elongate-triangular with the costa slightly arched, and a broad, doubly vent black streak in the disc near the base (Figure 2.1 A); the hind wings are mottle brown, speckled with a darker grey (Hudson 1928, Gaskin 1966) Considerable variations exist in the depth of the ground colour and females are usually lighter than males (Hudson 1928).

Gaskin (1966) proposed the name "the hood-marked bell moth" and more recently Steven (1990) gave the name "black-lyre leafroller" for *C. jactatana.* These names are derived from the conspicuous morphological characteristic of the species.

## 2.4 Distribution and Hosts

*Cnephasia jactatana* is widely distributed in the North and South Islands of New Zealand (Hudson 1928, Gaskin 1966, Crowe 2002), being particularly common in the Wellington area (Gaskin 1966). It is associated with forest, scrub country, bush environments and apple and kiwifruit orchards (Hudson 1928, Gaskin 1966, Steven 1990, Crowe 2002).

Several fruit and fibre crop plants have been reported as host plants of *C. jactatana*, e.g. *Actinidia deliciosa* (kiwifruit), *Vitis vinifera* (grapes), *Rubus idaeus* (raspberry), *Citrus*, *Crataegus*, *Diospyros kaki* (persimmon), *Eucalyptus*, *Malus domestica* (apple), *Pinus radiata, Prunus domestica* (plum) and *Phormium tenax* (New Zealand flax). It has also been recorded from plants surrounding the orchard as shelter belts such as *Salix fragilis* (crack willow), *Populus nigra* (Lombardy poplar), *Tribolium repens* (white clover), *Juglans regia* (walnut), *Ulnus glabra* (elm), among others (Gaskin 1966, Spiller and Wise 1982, Green 1984, Steven 1990, van der Geest et al. 1991, Suckling et al. 1998, Crowe 2002).

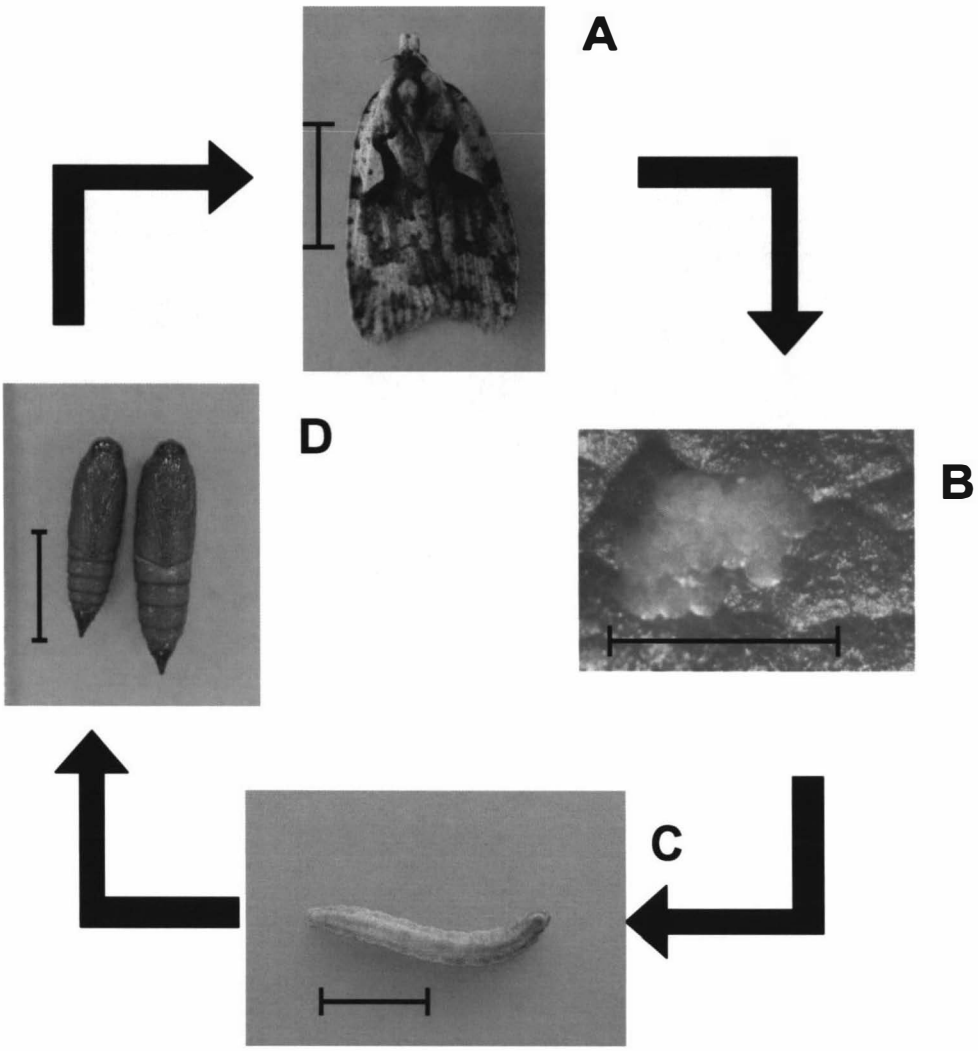


**Figure 2.1.** Life cycle of *Cnephasia jactatana.* A, adult moth with characteristic S-shaped mark on each of the front wings; B, eggs on kiwifruit leaf; C, mature larva and D, male (left) and female (right) pupae. Scale line = 5 mm.

## 2.5 General Biology

*Cnephasia jactatana* develops through four stages: egg, larva, pupa and adult. The majority of knowledge on the larval stages of this insect comes from the research by Ochieng'-Odero (1990a, 1990b, 1991, 1992) and Ochieng'-Odero and Singh (1992) who reared *C. jactatana* on an artificial diet in order to establish a laboratory colony.

### 2.5.1 Eggs

Tortricid eggs are dome-shaped with the long axis horizontal and the microphyle at one end. The chorion may be smooth, irregularly rugose, or reticulate with pronounced ridges (Horak 1991). Species of the tribe Archipini lay their eggs in regular imbricate masses (Powell and Common 1985). Under field conditions, *C. jactatana* eggs measure (mean ± SE) 0.73 ± 0.01 wide by 0.98 ± 0.01 mm long and the dorsal surface of the egg masses has a well defined polygonal pattern (Green 1984) (Figure 2.1B). Ochieng'-Odero and Singh (1992) reported an egg incubation period of > 14 days for *C. jactatana.*

### 2.5.2 Larvae

The tortricid caterpillar has primary and secondary setae only. Its body is divided into a very sclerotized head, a thorax with three pairs of segmented legs and an abdomen with five pairs of unsegmented prolegs (Horak 1991) (Figure 2.1 C). *Cnephasia jactatana* larvae can be differentiated from those of other leafrollers by the following characteristics: a) the head capsule has a gold-brown tinge and no ring around ocellus III, b) D1, D2 and SD1 setae arise from separate pinnaculae and c) the body of $L_4$ and $L_5$ is pinkish/brown with three dorso-lateral longitudinal stripes (Green 1984, N. Mauchline, pers. commu.).

When reared on the general purpose artificial diet (Singh 1983), *C. jactatana* took between 29 to 50 days to develop through five instars at 20 ± 1°C, 75 ± 5% RH under a photoperiod of 18:6h L:D (Ochieng'-Odero and Singh 1992). Bathon et al. (1991) reported a larval period of 39 days on the same diet at 20°C.

The mature ($5^{th}$ instar) *C. jactatana* larva is approximately 1.5 cm long (Figure 2.3) (Gaskin 1966, Steven 1990) and has a head capsule width of (mean ± 95% confidence interval) 1.19 ± 0.02 mm for males, and 1.24 ± 0.01 mm for females, and a larval critical weight ($L_{cw}$) of 29 and 36.4 mg for males and females, respectively (Ochieng'-Odero 1990a). The $L_{cw}$ is the weight above which, normal process of pupation or moult occurs (Ochieng'-Odero 1990a).

Temperature and photoperiod affect the development of *C. jactatana* larvae. An inverse linear relationship was found between final instar length and temperature.

For example, when reared at 15°C and 25°C the final instar length was 9 and 3 days, respectively, producing heavier insects at lower temperatures. When reared under different light regimes, an increase of the photophase length decreased the larval duration but did not modify larval weight. Shorter larval duration and heavier pupae were obtained when the rearing conditions imitated the natural conditions (photoperiod coinciding with thermoperiod) (Ochieng'-Odero 1991). No diapause has been reported for this species, but *C. pumicana* and *C. longana* enter diapause at the larval stage (Glas 1991).

*Cnephasia jactatana* larvae are polyphagus (J. S. Dugdale, pers. commu.) and may feed upon living and dying plant material (van der Geest et al. 1991, Wearing et al. 1991). They are often found on kiwifruit leaves around the fruit and within the beak of the fruit, either damaging the skin or burrowing into it. As fruit matures, it becomes more suitable to *C. jactatana* larvae and as a result more deep-flesh damage is caused by this species than any other leafroller species (Steven 1990, Stevens et al. 1995). In natural conditions, larvae of the closely related species *C. pumicana* and *C. longana* are extremely cannibalistic (Glas 1991).

### 2.5.3 Pupae

Tortricid pupae have no mandibles and appendages are fused to the body wall. They have 10 abdominal segments, with segments 4-6 in the female and segments 4-7 in the male being movable. Pupae are covered with spines, which are more developed in the male. *C. jactatana* pupae have two rows of < 10 spines each on the dorsal second abdominal tergite. Dark tipped spines exist in the anterior rows and are larger than those of the posterior (Green 1984) (Figure 2.1 D). Tortricid females and males are different at the pupal stage in the position of the anus and genital openings. Segment 8 in the female is strongly narrowed medially and its oblique caudal margins connect towards the copulatory opening (Horak 1991). Female pupae of *C. jactatana* (32-44 mg) are generally heavier than males (29-33 mg) (Bathon et al. 1991, Ochieng'-Odero and Singh 1992).

As with the larvae, the size of *C. jactatana* pupae is affected by environmental conditions and follows a similar trend to the larval stage. For example, pupae reared at 15°C are heavier than those reared at 20 or 25°C

(Ochieng'-Odero 1991). Insects reared at 20 ± 1°C, 75 ± 5% RH and a photoperiod of 18L:6D spend between 15 and 20 days as pupae (Ochieng'-Odero and Singh 1992).

### 2.5.4 Adults

Tortricid females emerge with few or no mature eggs in their ovaries (Benz 1991). Under laboratory conditions *C. jactatana* female and male adults live 19 - 29 days and 14 - 21 days, respectively (20°C, 75% RH, 18L:6D) (Ochieng'-Odero and Singh 1992). Adult weight and fecundity vary according to the rearing conditions: heavier pupae and more fecund females are produced at lower temperatures (Ochieng'-Odero 1992). The fecundity of females mated to average weight males (30 mg) at 20°C, ranged from 100 to 272 eggs (Ochieng'-Odero 1990a). He also reported that for each gram of weight at pupal stage 7.83 eggs are produced by the adult. However, due to a low mating rate, the regression values reported by Ochieng'-Odero (1990a) are gathered from 60 pairs from three different generations, jeopardizing the accuracy of the weight-fecundity relationship.

Different photoperiods (18:6, 12:12, 6:8 and 0:24 h L:D) at 18° C have no effect on the female fecundity. Fertility rate and sex ratio reported throughout 12 laboratory generations range from 55 to 68% and 1:0.7 to 1:1.08 ♂:♀, respectively (Ochieng'-Odero and Singh 1992).

Ochieng'-Odero (1990a, 1990b, 1991, 1992) and Ochieng'-Odero and Singh (1992) did not clearly state the food source provided to *C. jactatana* adults. However, Ochieng'-Odero and Singh (1992) mentioned that their rearing system was similar to that used by Singh et al. (1985) for rearing *Epiphyas postvittana*, where a 10% honey solution was used to feed the adult.

## 2.6 Reproductive Biology of Tortricidae

### 2.6.1 Female and Male Reproductive Systems

The tortricid female reproductive system presents two sexual openings: the ovipore, through which eggs are oviposited, and the copulatory orifice. There are eight ovarioles divided into two groups. Mature eggs move from the ovarioles to the

calyx and thence pass through the lateral oviduct toward the common oviduct. Fertilization occurs at the vestibulum level. The uterus joins the vestibulum and the common oviduct with the ovipositor. The ductus bursae joins the copulatory orifice with the bursa copulatrix where spermatophores are deposited. The bursa seminalis is connected by a seminal duct to the bursa copulatrix, and by a 2$^{nd}$ seminal duct to the vestibulum. A sex pheromone gland is situated at the base of the ovipositor in the intersegmental region between the eighth and ninth segments (Benz 1991).

The tortricid male reproductive system is fully formed and mature at the pupal stage. During the larval/pupal metamorphosis the testes fuse to a single round structure surrounded by a red, brown, violet, or bluish envelope called scrotum connected to a vasa deferentia. The vasa deferentia has two pairs of seminal vesicles serving as sperm-storing organs. The second seminal vesicle joins the ductus ejaculatorius duplex which becomes the ductus ejaculatory simplex, and ends at the aedeagus. Tortricid males produce two types of sperm, the anucleated infertile sperm called apyrene and the nucleated fertile sperm called eupyrene. Production of both sperm types occurs as early as in the pupal stage (Benz 1991). In some genera of the Archipini, adult males possess pheromone glands at the tip of the abdomen, on hind wings or hindlegs [e.g. *Cryptophlebia leucotreta* (Zagatti and Castel 1987, Benz 1991)]. These structures produce a pheromone blend to attract females at close range and are released during courtship display (Benz 1991).

### 2.6.2 Mating Behaviour

No information on the mating behaviour of *C. jactatana* is currently available. The mating behaviour of Tortricidae is very diverse and is specific for each species. In general, mating starts when the female calls the male by everting the posterior end of the last abdominal segment exposing the opening of the pheromone gland, and raising the abdomen or vibrating the tip of the abdomen (Benz 1991). The *Cydia pomonella* female presses her thorax close to the substrate, extends and elevates antennae, raise and extend the abdomen in a down-curved position at the same time protruding the ovipositor to expose the pheromone gland (Howell 1991). By contrast, the European grape berry moth, *Eupoecilia ambiguella*, curves the abdomen upwards exposing the pheromone gland (Schmieder-Wenzel and Schruft 1990). The male curls the end of his abdomen upwards whilst fanning his wings,

walks up to the female and touches her with his head. The male curves his abdomen laterally and touches the female with the tip of his abdomen. If the female accepts the mating attempt, she raises her wings and exposes her abdomen. Both sexes remain motionless during copula (Benz 1991). Copula length varies from 40-60 minutes in *Cy. pomonella* (Ferro and Akre 1975) to >1 hour in *Strepsicrates macropetana* (Mauchline 2000).

### 2.6.3 Insemination and Fertilization

During mating, the tortricid male introduces the aedeagus through the copulatory orifice into the bursa copulatrix where the spermatophore(s) is (are) deposited. The transfer of one spermatophore per mating is the rule. Sperm is released into the bursa copulatrix and travels to the bulla seminalis aided by contractions of the seminal duct and the oviduct. Sperm travels to the vestibulum and thence to the spermatheca. In order to fertilise the eggs, sperm must travel from the spermatheca downwards via spiral duct to the vestibulum where fertilisation takes place (Benz 1991). In *Cy. pomonella*, sperm needs between 3 and 6 hours to exit the spermatophore (Howell 1991). In most Lepidoptera oviposition begins < 24 h after mating. In Tortricidae and other groups, spermatophores remain in the females' body and can be used to indicate the number of matings achieved by a female (Drummond 1984, Benz 1991, Howell 1991).

In most tortricid species transfer of a spermatophore is necessary for egg fertilisation (Benz 1991). However, multiple-mated *G. molesta* and *Lobesia botrana* males are able to fertilise eggs without transferring a spermatophore (George and Howard 1968, Torres-Vila et al. 1995).

### 2.6.4 Reproductive Activity Patterns

#### 2.6.4.1 Daily Activity Patterns

The rhythm of reproductive activity has been studied in several tortricid species. For example, Chambon (1976) studied the daily activities of *C. pumicana* under natural conditions. Adults spend all day resting on grasses and fly to woods at 2030 hours. This flight period ends at 2200 hours and the females begin to oviposit on tree branches and trunks; the oviposition period is completed at approximately

0300 hours. A second flight period is observed at 0400-0700 hours, during which mating occurs frequently, ceasing at dawn.

#### 2.6.4.2 Lifespan Activity Patterns

Lawrance and Bartell (1972) and Gu and Danthanarayana (1990a) provided information on the activity patterns of the light-brown apple moth, *E. postvittana*, throughout their lifespan. Under laboratory conditions, females as young as 1 d old call for mates. Similarly, newly-emerged males are sexually interested. However, very few moths (3%) mate within 24 hours after emergence and more than 40% of moths mate when 3 days old. Oviposition takes place within 24 hours after mating and peaks 4 days after emergence.

## 2.7 Factors Affecting Reproductive Fitness

A great effort has been dedicated to the study of the influence of abiotic and biotic factors on insect reproductive performance. In this section, I will review the effect of body weight, longevity, age, larval diet and mating on female and male reproductive fitness. These factors interact and affect both sexes' reproductive fitness to different degrees.

The amount of scientific literature describing the effects of a plethora of factors on female fecundity is vast. Most papers focus on the female reproductive output (e.g. Danthanarayana 1975b and 1983, Ellis and Steele 1982, Vahed 1998, Bergström et al. 2002, Tregenza and Wedell 2002), neglecting the influence of the male on the female reproductive fitness. Some publications, however, have addressed the effect of male mating history on female fecundity (e. g. Halliday 1987, Carroll 1994, Delisle and Bouchard 1995, Gage and Cook 1994, Tammaru et al. 1996a and 1996b, Wedell 1996). Abiotic factors during larval growth that affect the reproductive output of *C. jactatana* have been mentioned in Section 2.5.4.

### 2.7.1 Effect of Mating on Insect Physiology

Mating is a turning point in the insect's life that modifies its physiology and behaviour, and determines its future actions. Mating is crucial for the survival and evolution of the species because it is the time in the life of the species when

transference of genetic material is made. The timing of mating modifies the ability of both sexes to obtain further partners and influences the genetic quality and survival probabilities of the offspring.

In many insect species, a male transfers enough sperm to fertilise the full egg-load of a female in a single ejaculation (Thornhill and Alcock 1983, Drummond 1984). Tortricid spermatophores contain sperm and male-derivate substances (MDS) responsible for the change in physiology and behaviour of females (Gillott 2003). MDS play an important role in the induction of ovulation, ova maturation, regulation of egg development, and oviposition, and protection of eggs from predators. They trigger female's modulation of host seeking behaviour and circadian rhythmicity. Further, MDS may affect sperm survival in the female's reproductive tract, provide sperm nutrition, pH buffering, osmotic buffering and defences against oxidising agents (Davey 1985, Ridley 1988, Eberhard and Cordero 1995, Karube and Kobayashi 1999, Gillott 2003). In addition, MDS can be incorporated into the female soma to boost fecundity and longevity (Wiklund et al. 1993, Gillott 2003).

Female physiology and behaviour are modified by the presence of sperm and MDS in her reproductive system. After mating, females may become permanently or temporarily less receptive or attractive to males or their behaviour may become directed to seeking out oviposition sites (Davey 1985). On the other hand, unmated females of many species, which develop a full complement of mature eggs, lay only a portion of them and the rest may be reabsorbed and used for body maintenance (Davey 1985). For example, unmated *E. postvittana* females do not start to lay eggs until they are 6 days old and then they oviposit them in an erratic pattern. In contrast, *E. postvittana* females mated when 2 d old oviposit a substantial number of eggs within one day of mating, and continue at a steady rate over the next 6 d, producing significantly more eggs than unmated females (Danthanarayana and Gu 1991, Foster and Howard 1999).

The mating process requires energy resources for reproductive and pre-reproductive activities such as searching for mates, defending a territory, chasing away competitors, etc. All of these high energy-demanding pre-reproductive activities require resources that cannot be invested in somatic maintenance or reproductive effort. There is little direct evidence that individuals reach physiological

limits during a single bout of the above-mentioned pre-reproductive behaviours, but over a longer timeframe they may deplete the resources available. If intensity and endurance required to perform a specific behaviour confer an advantage in terms of mating success, then the energy available for the behaviour can limit the number or the rate at which males can engage in sexual activities (Halliday 1987).

In addition, mating has other physiological consequences for males. For example, after males have mated, they produce smaller spermatophores (Royer and McNeil 1993, Delisle and Bouchard 1995) containing fewer sperm (Royer and McNeil 1993, Ofuya 1995, Cook 1999). They also have to invest more time in subsequent matings (Kaitala and Wiklund 1995, Bissoondath and Wiklund 1996a, Sakurai 1998), and their remating opportunities diminish as females may choose a fitter male for mating.

Mating has serious physiological and reproductive consequences for already mated females. They are not attractive to males and have fewer opportunities to remate in the presence of virgin females (Lingren et al. 1988, Spurgeon et al. 1995, Proshold 1996), reducing the probability for increasing the genetic diversity of their progeny. Mating also reduces the females' lifespan (Partridge 1987, Keller and Reeve 1995, Fadamiro and Baker 1999, Hou and Sheng 1999, Kawagoe et al. 2001), as well as their time for feeding and oviposition (Keller and Reeve 1995). In turn, these costs may decrease female egg production (Arnqvist and Nilsson 2000).

Mating may also increase the risk of predation as both insects are more exposed to natural enemies during mating (Rowe 1994, Bissondath and Wiklund 1996a). During mating, females may suffer physical damage (Helversen and Helversen 1991) and cross-infection from parasites or pathogen (Hurst et al. 1995, Rolff and Siva-Jothy 2002). Highly promiscuous males are more susceptible to bacterial infections, in turn affecting their longevity (Mckean and Nunney 2001).

In many species, males continuously court females to the point where they are prevented from oviposition or feeding. Females may accept an extra copula if it is less costly in terms of time, than resistance and continuous rejection of male solicitation (Drummond 1984, Cook 1999). Continuous cohabitation of males and

females tends to increase the mating rate and thus the cost of mating (Arnqvist and Nilsson 2000).

### 2.7.2 Effect of Bodyweight and Longevity on Reproductive Fitness

Sexual selection assumes that one or more components of fitness are a function of increasing body size (Roff 1992, Andersson 1994), through an allometric or linear relationship (Klingenberg and Spence 1977). Body size/weight has traditionally been considered a key determinant of an organism's ecological and physiological properties (Thornill and Alcock 1983, Honěk 1993), because this is strongly correlated with many physiological and fitness characters (Reiss 1989, Roff 1992). Female weight is generally accepted as an index of potential fecundity, assuming a positive relationship between the number of oocytes in the ovarioles and the weight of the female. Therefore, heavy females have more and larger eggs available and are able to regenerate eggs faster when required (Danthanarayana 1975b, Carroll and Quiring 1993, Torres-Vila et al. 1999).

In males, large size/weight has been used as an indication of "good quality", such as having better genes, more sperm, larger nuptial gifts, and greater ability to overcome female rejection or a better chance to win in male-male competition (Phelan and Barker 1986, Thornhill and Alcock 1983, Honěk 1993, Andersson 1994, Vahed 1998). For example, in the tortricid *Zeiraphera canadensis*, large males transfer a 30% larger spermatophore than small males (Carroll 1994).

Similarly, large bodyweight/size has been associated with greater longevity and extended oviposition period, and therefore higher lifetime fecundity. The general view is that large insects have a better chance to survive either by winning food sources, displacing small individuals or due to larger energy reserves. Longevity may affect reproductive fitness, as some females may die shortly after emergence, after mating or before oviposition has occurred (Partridge 1987, Šmits et al. 2001).

However, there is a growing list of studies reporting no effect or even negative effects of weight/size on fitness components (Leather and Burnand 1987, Svärd and Wiklund 1988). For example, Carroll and Quiring (1993) failed to find any size-related effects on fecundity and longevity on *Z. canadensis*. An interesting report from Marshall (1990) shows that the reproductive effort of the pyralid

*Parapediasia teterrella* is positively associated with the female body weight at or below the average but negatively associated with weight above the average of the population. These reports suggest that the 'larger-the-better' hypothesis does not apply to every species, and that there are some trade-offs between the interaction of bodyweight, longevity and fecundity.

In order to attain a large bodyweight/size, individuals must acquire enough resources to grow large and to remain competitive within the population. Some authors have pointed out the reasons why organisms cannot grow indefinitely (Roff 1981, Blanckenhorn 2000, Thompson and Fincke 2002), and why it is advantageous to attain an optimal size (Roff 1981, Tanaka 1981).

Blanckenhorn (2000) reviewed the proposed mechanisms that regulate body size in multicellular organisms. These procedures are present throughout the life of an individual and their effects can be seen at any time. In order to achieve a larger size, an individual can either increase its development rate or extend its pre-reproductive period. Increasing the pre-reproductive period increases the cumulative mortality before reproduction due to predation, parasitism and/or starvation. Increasing the developmental rate increases the chance of mortality due to predation associated with the riskier foraging necessary to supply food for a faster metabolism. Also, large individuals are more visible, and less agile and manoeuvrable and may suffer more heat stress than small ones. This situation may result in selective predation on larger organisms. Larger individuals in good condition may reproduce early and show greater reproductive effort and therefore are expected to suffer a greater cost of reproduction (Partridge 1987, Blanckenhorn 2000).

### 2.7.3 Effect of Diet on Bodyweight, Fecundity and Longevity

Female fecundity and longevity are associated with food consumed during the larval and adult stages. The quality and quantity of food available to the larvae determine the adult energy reserves available for reproduction and body maintenance (Torres-Vila et al. 1999, Stjernholm and Karlsson 2000). For example, in the geometrid *Epirrita autumnata* females developed under food stress are smaller and show reduced fecundity compared to well-fed females (Tammaru et al. 1996b). Food stressed females may have a reduction in essential compounds for production or

maturation of eggs (Watt 1986). Larvae of *E. postvittana* reared on different hosts produce adults of different weights, fecundities and longevities (Danthanarayana 1975a, 1975b). Similar results have been reported for other tortricids (Cisneros and Barnes 1974, Savopoulou-Soultani et al. 1990, Delisle and Bouchard 1995, Delisle and Hardy 1997, Torres-Vila et al. 1999).

As with females, male fecundate capacity is affected by the quality and quantity of food available to the larvae. In many cases, males reared under food stress or in crowded conditions are smaller and have a reduced fecundate capacity due to a diminished sperm production, or may provoke a shorter female refractory period. For example, *Ch. fumiferana* females mated to males developed on old balsam fir foliage resume calling and remate earlier than females mated to males reared on young balsam fir foliage, a better quality larval food (Delisle and Hardy 1997). Similarly, *Ch. rosaceana* males reared on a low-quality food are unable to remate at a similar rate to those reared on maple (*Acer* sp) or artificial diet (Delisle and Bouchard 1995).

The importance of adult food resources on reproduction depends on adult feeding habits and timing of egg maturation (Murphy et al. 1983, Boggs 1986, Wheeler 1996). Female fecundity may be affected by the quality and quantity of food supplied during the adult stage (Wheeler 1996). This food may include water, nectar, honey, mixtures of sugars, mixtures of sugar and protein, and MDS transferred during mating. According to Benz (1991), most adult tortricids require only water for normal longevity and fecundity. Species like *L. botrana*, *G. molesta*, *Cy. pomonella* and *Pandemis heparana* require only water for normal reproduction (Howell 1981 and 1991, Savopoulou-Soultani et al. 1998). Carbohydrates, as sucrose or honey, or a mixture of carbohydrates and protein, have different effects on adult fecundity and longevity. For example, *Homona coffearia* females need sugar in order to produce eggs (Benz 1991). Sucrose ingestion by *Z. canadensis* increases female longevity and fecundity (Carroll and Quiring 1992), and *Epinotia aporema* females fed with 10% honey solution produce 15 times more eggs than water fed females (Benz 1991). However, Gu and Danthanarayana (1990b) reported no change in fecundity in *E. postvittana* females fed with water or 30% honey solution. Similarly, adult food

containing protein did not improve the fecundity of *L. botrana* (Stavridis and Savopoulou-Soultani 1994 cited in Savopoulou-Soultani et al. 1998).

Adult food supply can affect longevity without affecting female reproductive output. For example, *Cy. pomonella* females fed on a carbohydrate solution or a carbohydrate-protein solution present similar fecundity, mating rate and fertility rate to water fed females, regardless of an increase in longevity (Howell 1981).

Reports by He and Tsubaki (1992) and He and Miyata (1997) for the noctuid *Pseudaletia separata* contrast with the general trend outlined above. In this species, small males produce a larger spermatophore than large males. In addition, males from a crowded rearing system produce significantly more apyrene sperm than those reared individually.

### 2.7.4 Effect of Mating Delay on Reproductive Fitness

Sexual fitness is affected by the age at which insects mate because mating triggers gonadic maturation in females and reduces fecundate capacity in males. In general, insects mated when sexually mature achieve better reproductive performances than insects mated at any other time. However, males and females may require different times to reach maturity (Benz 1991, Howell 1991).

Reports show that mating delay is generally detrimental to the reproductive fitness of insects. These adverse effects can be a shortening of the oviposition period, reduction in fecundity and fertility, and an increase in mating length. For example, *E. postvittana* females mated when 8 d old shorten their oviposition period by 4 days compared to females mated when 2 d old (Foster and Howard 1999). Delaying mating for 2-4 d in *Cy. pomonella* causes a 25-50% reduction in female fecundity and fertility, respectively (Karalius and Buda 1995). Old *Grapholita molesta* males invest more time in copula and present reduced fertility than middle age males (Delisle 1995).

Mating delay in females may reduce their reproductive fitness by allowing oviposition of unfertilised eggs by virgin females (Foster et al. 1995) or decreasing attractiveness of old females to potential mates (Lingren et al. 1988, Spurgeon et al. 1995, Proshold 1996). For example, *E. postvittana* females > 2 d old are expected to

have fewer opportunities to attract a mate due to a reduction of pheromone production (Foster et al. 1995).

Mating delay in males may also reduce their reproductive fitness (Howell 1991). For example, mating delay in *E. postvittana* males reduces their mating success. Three-d-old males have greater opportunity to locate a calling female due to a faster response to the female sex pheromone than 5–d-old males (Foster et al. 1995). Similar results were reported by Howell (1991) for *Cy. pomonella* and Delisle (1995) for *Ch. rosaceana*. In the latter species, >3-d-old males have a reduced probability of mating than 3-d-old males.

Mating delay in males may also affect fertility because of a limited amount of sperm available daily for fertilisation (Saunders 1982, Halliday 1987). In the gelechiid *Pectinophora gossypiella*, sperm is stored and accumulated at the ductus ejaculatoris until mating occurs (LaChance et al. 1977), suggesting that males that have no access to females would accumulate a greater amount of sperm and be able to transmit a larger ejaculate. Similarly, the longer the time between emergence and mating is, the larger the spermatophore is transferred by pierid *Pieris rapae* (Wedell and Cook 1999b). However, sperm reabsorption in old virgin males would result in a normal supply of sperm available for fertilisation (Wedell et al. 2002).

### 2.7.5 Female Multiple Mating

The effect of multiple mating on female reproductive fitness has been reviewed by many authors (e.g. Thornhill and Alcock 1983, Drummond 1984, Ridley 1988, 1990, Vahed 1998, Keller and Reeve 1995, Jennions and Petrie 2000), and is one of the most active fields of study in insect mating behaviour.

Females can mate one or more times during their lifespan depending on their mating systems (Ridley 1988). In many insect species a female obtains enough sperm from a single mating to fertilise her full egg-load (Thornhill and Alcock 1983, Drummond 1984, He and Miyata 1997). Nevertheless, many females mate more than once, either with the same male (repeated mating) or with different males (multiple mating) (Ridley 1990, Arnqvist and Nilsson 2000).

Several hypotheses have been proposed to explain this female re-mating behaviour, and these can be divided into two general classes: 1) material benefits and/or 2) genetic benefits (Reynolds 1996, Jennions and Petrie 1997). The material benefits of remating may include nutritional resources in the form of nuptial gifts, and replenishment of sperm where one mating provides insufficient sperm to fertilise a females' egg load (Newcomer et al. 1999); females may remate to collect an adequate amount of sperm and gonadotropins to maintain high production of viable eggs throughout her life (Arnqvist and Nilsson 2000), and to reduce the cost of long-term sperm storage (Drummond 1984). Genetic benefits of multiple mating include the avoidance of inbreeding (Madsen et al. 1992, Keller and Reeve 1995, Tregenza and Wedell 2002), manipulating offspring paternity (Andersson 1994, Edvardsson and Arnqvist 2000), reducing chances of fertilisation by sperm that is genetically defective due to age (Halliday and Arnold 1987), production of sons with genotypes that make them more attractive (Andersson 1994) or diminishing incompatible genotypes (Zeh and Zeh 1996).

The effect of multiple mating or remating on fecundity is highly dependant on the mating system of each species (Ridley 1988). Several studies have found a positive relationship between the number of matings and female fecundity (Danthanarayana and Gu 1991, Pardo et al. 1995, Ward and Landolt 1995, Sakurai 1996, Oberhauser 1997, LaMunyon 1997, Wilson et al. 1999), while others did not show such a relationship (Kraan and Straten 1988, Svärd and Wiklund 1988, Ono et al. 1995, Rodríguez 1998, Kawagoe et al. 2001), or even found a negative association (Cook 1999).

Whether a female also remates depends on several other factors. In species where adults feed on a high protein diet or have a short lifespan, remating has little effect on female fecundity. However, in species where adult females do not feed on a high-protein diet, emerge with no or few mature eggs, have a long lifespan, are reared on a low quality larval diet, and males transfer both sperm and MDS, remating has a great impact on female fecundity (Ridley 1988, Boggs 1990).

Remating in a monoandrous species (in which a female normally mates once in her life) is possible if females are exposed continuously to males. However,

remating is not expected to increase fecundity because males are selected to transfer enough sperm at a single mating to fertilise all the eggs (Ridley 1988).

Female remating may be necessary if the first mating fails to pass fertile sperm, or fails to transfer enough sperm (Ridley 1988). For example, females of the pierid, *P. napi,* receiving large ejaculates remate later than females receiving small ones (Kaitala and Wiklund 1995, Wiklund and Kaitala 1995).

The quality of the second ejaculate obtained by a female insect may influence her fecundity. Females receiving a second ejaculate from virgin males generally have higher fecundity than females receiving an ejaculate from a non-virgin male (Wiklund et al. 1993, Oberhauser 1997, Karlsson 1998, Makee and Saour 2001). However, in the noctuid *Spodoptera litoralis* (Sadek 2001) receiving a second ejaculate from a virgin male does not increase female fecundity.

Female remating can be influenced by the male's mating history and female size. For example, large females of the pierid *P. rapae* mated to virgin males receive more sperm and have longer refractory period than small females, but require more matings to fulfil their sperm needs (Wedell and Cook 1999a).

Female remating behaviour can be influenced by the time invested in copula. It is reported that long matings may result in the transference of larger amounts of sperm and MDS, consequently larger spermatophores (Rutowski et al. 1987). Therefore, short matings have been considered a reason for remating (Kaitala and Wiklund 1995, Bissoondath and Wiklund 1996a, Sakurai 1998). In *E. postvittana*, however, spermatophore size seems to play no role in the remating behaviour of females mated to virgin females. Female remating behaviour relates to the quality of the first mating as females mated to previously mated males have a reduced fecundity and are more likely to remate with an increase in the number of previous matings by the males. Similarly, females mated to previously mated males were sexually receptive earlier than females mated to a virgin male (Foster and Ayers 1996, Foster and Howard 1999).

### 2.7.6 Male Multiple Mating

Some research suggests that male ejaculate is inexpensive and unlimited and its only function was to fertilise the ova (Thornhill and Alcock 1983) but male ejaculate is not cheap and contains MDS that modify the female's behaviour and physiology, and may be used for somatic maintenance or to enhance fecundity (Halliday 1987, Wiklund et al. 1993, Bissondath and Wiklund 1996a and 1996b, Gillott 2003). Production of ejaculate and MDS is costly (Svärd and Wiklund 1986a), dynamic in time and space (Giebultowicz and Brooks 1998, Wedell et al. 2002) and limited (Svärd and Wiklund 1986b, Royer and McNeil 1993, Giebultowicz and Brooks 1998). For example, insects that feed in the adult stage on a low protein diet must sequester most of the protein needed for gamete production and maintenance during their larval feeding (Gage and Cook 1994, Kaitala and Wiklund 1995). Production of sperm and MDS is constrained by the energy reserves of the insect (Gage and Cook 1994, Kaitala and Wiklund 1995) and the speed of transforming storage material into gametes or MDS (Drummond 1984, Bissoondath and Wiklund 1996a). Nutrients contained in the ejaculate have been found in the eggs and soma of females (Wiklund et al. 1993), demostrating their importance for the female reproductive output.

Traditionally, it has been assumed that the size of a spermatophore is directly related to its reproductive value (Marshall and McNeil 1989) but the relationship between spermatophore size, ejaculate quality and fecundate capacity in Lepidoptera is not always positive and direct. Spermatophore size may be affected by male body size (Carroll 1994, Wiklund and Kaitala 1995), age at mating (He and Tsubaki 1992), mating history (Royer and McNeil 1993, Delisle and Bouchard 1995, Cook 1999) and time between successive matings (Svärd 1985, Svärd and Wiklund 1986a and 1989). These variables modify the nutritional value and fecundate capacity of the spermatophore. Generally, large males produce larger ejaculates (Carroll 1994, Wiklund and Kaitala 1995) and are able to produce a second ejaculate earlier (Carroll 1994) or larger (Wiklund and Kaitala 1995) than small males. However in the pierid *P. napi*, this relationship is valid only for the second ejaculate delivered within 24 hours. In this species the first spermatophore produced by males, irrespective of body size, is similar (Wiklund and Kaitala 1995). Further, in *P. rapae*, the large male

produces a larger spermatophore but the amount of fertile sperm is similar to that contained in a smaller spermatophore (Wedell and Cook 1999b).

### 2.7.7 Mate Choice, Sexual Selection and Mating Systems

Mate choice, sexual selection and mating systems have been reviewed by a number of authors including O'Donald (1980), Bateson (1983), Smith (1984), Bradbury and Andersson (1987), Andersson (1994), Jennions and Petrie (1997) and Choe and Crespi (1997). The number of publications on mate choice, sexual selection and mating systems in the animal kingdom is overwhelming. I will review some of those related to insects, particularly Lepidoptera.

#### 2.7.7.1 Mate Choice

Halliday (1983) defines mate choice as "any pattern of behaviour shown by members of one sex that leads to their being more likely to mate with certain members of the opposite sex than with others". The study of mate choice is linked to sexual selection, mating systems and evolution. These topics have raised concepts like nuptial feeding, parental investment, sperm competition, last-male sperm precedence, sexual conflict, mating control, etc.

Individuals choose their partners because potential mates vary in quality, quantity and availability. Each potential mate presents its own specific array of cost and benefits. It is thus in the best interest of the insect to gauge the quality of potential mates as accurately as possible before investing resources (gametes, time or MDS) (Halliday 1983). Mate choice is a dynamic process involving at least two individuals assessing the quality of each other simultaneously. Male-male or female-female interactions (intra-sexual selection) decide who has the chance to be a candidate to fertilise eggs while the ability of an individual to attract members of the opposite sex (inter-sexual selection) decides who has the opportunity to select or being selected (Jennions and Petrie 1997, Panhuis et al. 2001). Mate choice is also influenced by the spatial distribution of potential mates, the operational sex ratio (OSR, average ratio of fertilisable females to sexually active males at a given time), the time available for sampling potential mates, the distribution of resources in the environment and the willingness of an individual to invest its resources (gametes

and/or MDS) in an specific mate (Emlen and Oring 1977, Halliday 1983, Alexander et al. 1997, Jennions and Petrie 1997).

Darwin (1859) suggested that females should be more discriminating than males in their choice of mates. The rationale is that eggs cost more to produce than sperm and females as general rule mate fewer times than males. This leads to the conclusion that females have more to lose from suboptimal matings than males do. So females should be highly selective and males should suffer the effects of sexual selection (Alexander et al. 1997). However, when sperm is a scarce resource due to variation in male quality or availability, males are the selective sex, and females compete for males and suffer the effects of sexual selection (Petrie 1983, van Dongen et al. 1998, Bonduriansky and Brooks 1998). Such species are called reverse-role species (Petrie 1983). Selectiveness positively correlates with attractiveness, therefore, less attractive individuals are less selective than attractive ones (Jennions and Petrie 1997). For example, less attractive females may become less selective than more attractive females when the population is female-biased (Jennions and Petrie 1997).

Six major forms of general mate assessment strategies have been proposed for mate choice: a) random mating: an individual accepts the first mate encountered; b) threshold-comparison: a mate that exceeds some threshold level for the character of choice is selected; c) sequential comparison: an individual compares mates until the most recently encountered is of lower quality than the previous one, and then accepts the previously encountered mate; d) one-step decision or sequential search: the accepted mate exceeds the average population quality; e) The "best of N-rule" or pooled comparison: an individual can sample and compare several potential mates and select the "best" of them; and f) the best of all: an individual selects the mate with the optimal cue value among many encountered in a finite time (Svensson 1996, Alexander et al. 1997, Jennions and Petrie 1997). The best-of-all model yields the best result for the females but requires that females have plenty of time and can return to any male that they have sampled. Such conditions are probably rare in natural conditions (Halliday 1983).

The preceding proposals assume that females mate once and the males' fitness does not change as the number of matings increases (Halliday 1983).

However, the assumption may not be valid as male fecundate capacity is limited (Halliday 1987, Gillott 2003).

#### 2.7.7.2 Sexual Selection

Sexual selection refers to the selection of characters that give certain individuals a mating advantage over others of the same sex (Breuker and Brakefield 2002). For example, when males access receptive females by winning a physical battle, size and weaponry confer significant advantage, but when mating success depends on a non-physical competition between males, energy reserves and efficiency may be more relevant (Kemp 2002).

Classical sexual selection predicts that young, virgin and large mates should be preferred because they ensure offspring with better characteristics and higher assurance of paternity (Halliday 1983, Andersson 1994). Large individuals are able to produce more gametes, provide a larger nuptial gift, a larger territory or better oviposition sites in addition to their genetic quality (Phelan and Barker 1986, Honěk 1993, Bissoondath and Wiklund 1996a, Vahed 1998, Cloutier et al, 2000, García-Barros 2000). Young mates have a better reproductive output (Karalius and Buda 1995, Vickers 1997), and are more likely to be virgin, reducing sperm competition (Parker 1984).

Several authors have documented mating advantages associated with large (Carroll 1994, van Dongen et al. 1998), virgin (Lewis and Iannini 1995, Arnaud and Haubruge 1999) and young individuals (Yasui 1996). Nevertheless, in all three cases, there is evidence that for some species the classical sexual selection theory does not apply. For example in the tortricid *Ch. rosaceana*, old females obtain more matings than young females and old males spend less time in copula than young insects (Delisle 1995). In the butterfly *Bicyclus anynana* females prefer to mate with medium size males (Breuker and Brakefield 2002) and *Drosophila hydei* males prefer to mate with recently mated females (Markow 1985).

#### 2.7.7.3 Mating Systems

The study of mating systems deals with general strategies employed in obtaining mates. It includes such features like the number of mates acquired, the

manner of mate acquisition, the presence and characteristics of any pair bonds and the patterns of parental care provided by each sex (Emlen and Oring 1977).

The mating system of a species is not fixed in time or space because the ability of a sex to control key resources and the availability and distribution of potential mates, food, oviposition places, etc may vary from time to time (Choe and Crespi 1997). How insect nutrition, mate selection, etc. affect male and female reproductive output comprises the mating system of a species. The precise form of a mating system will depend on which sex is limiting and on the manner in and the degree to which the limited sex controls the resource and/or monopolises mates (Emlen and Oring 1977).

In species where sperm competition is high and female remating is governed by the amount of sperm storage in her body, males tend to inseminate the female with larger quantities of sperm. In species where female remating frequency is low, males tend to transfer smaller ejaculates. In general, the last male that copulates with a multiply-mated female sires most of the resultant offspring, a phenomenon known as last-male sperm precedent (Drummond 1984). However, in double-mated *Ch. fumiferana* females, the first male fathers all of the offspring (Drummond 1984). In the nymphalid *Limenitis arthemis-astyanax*, the offspring show mixed characteristics suggesting a partial or complete sperm mixture in the females' body (Platt and Allen 2001). In the pierid *P. napi*, larger males obtain the majority of fertilisations, and the degree of second-male sperm precedence is positively related to body size of mating males (Bissoondath and Wiklund 1997). All these differences in fertilisation success are due to the species mating systems.

In Tortricidae, the mating system of *E. postvittana* is relatively well understood. Males are sexually mature soon after emergence but most females are sexually receptive when > 1 d old (Lawrence and Bartell 1972, Gu and Danthanarayana 1990a). Mating stimulates oviposition while unmated females oviposit few eggs in an erratic pattern (Danthanarayana and Gu 1991, Foster and Howard 1999). Heavy females are generally more fecund and live longer than light females, but fecundity and longevity are related to the quality and quantity of the larval food, temperature and rearing density (Danthanarayana 1975a, 1975b, 1983, Tomkins et al. 1989). Addition of carbohydrates to the adult diet did not modify

fecundity of *E. postvittana* (Gu and Danthanarayana 1990b). Males mate 6.6 times on average while females are generally monoandrous. Recently mated females stop pheromone production and are not receptive (Foster and Roelofs 1994).

Therefore, the mating system of a species depends on the availability of resources in time and space and the capacity of a sex to monopolise the key resources (Emlen and Oring 1977). It also depends on the assessment of the quality of the potential partners and how male and female quality changes in time. Overall, the mating system of a species depends on a specific combination of abiotic and biotic factors.

# CHAPTER 3
# REPRODUCTIVE BIOLOGY OF *CNEPHASIA JACTATANA*

## 3.1 General Introduction

*Cnephasia jactatana* is a key pest of kiwifruit in New Zealand. Little information is available on the biology of this species. Most knowledge about this species was gathered during the process of the establishment of a laboratory colony by Ochieng'-Odero (1990a, 1990b, 1991, 1992) and Ochieng'-Odero and Singh (1992). The effect of environmental conditions on the physiology and duration of the larval stage was the core of their research. Their reports on the fecundity of this species are the only information currently available. Information about other aspects of the biology of this species is scattered. This chapter reports sexual maturation, circadian rhythms and reproductive potential of *C. jactatana.*

## 3.2 General Methodology

The materials, procedures, environmental conditions and definitions detailed in this Section were used throughout the thesis.

### 3.2.1 Materials

*Test cylinders*: Transparent plastic cylinders (65 diameter × 83 mm high, LabServ, Auckland) were used for rearing and experiments on reproductive behaviour. The container was lined with a multipore plastic film (Wicket bag plain perforated, 15μm, manufactured by Cryovac™, W.R. Grace Ltd, Auckland) as an oviposition substrate. The cylinder was covered with a plastic sheet secured with a rubber band, and 10% sucrose solution provided in a plastic tube (10 diameter by 40 mm long) was dispensed with a 3.75 cm cotton wick (Richmond, Charlotte, N.C., USA) inserted through the plastic sheet. The plastic tubes were refilled with 10% sucrose solution as needed. The insertion of the plastic tube through the plastic sheet allowed airflow.

*Glass vials*: The glass vials (15 by 50 mm, Samco, U.K) with 10% sucrose solution dispensed through a 3.75 cm cotton wick (Richmond, Charlotte, N.C., USA) inserted through the plastic lid were used for temporary rearing of individual adults between experiments.

*Dissecting microscope*: An Olympus SZ III (Japan) dissecting microscope with transmitted light fitted with a micrometer eyepiece was used for dissecting, counting and measurement.

*Electronic scale*: A Mettler electronic scale of 0.0001g accuracy (Type AE 100-S, SNR 55115, Zurich, Switzerland) was used for weighing pupae.

### 3.2.2 Procedures

*Egg incubation*: Egg masses were collected daily and placed on a damp filter paper (Whatman No.1, 42.5 mm diameter; Whatman International Ltd, Maidstone, England) in the bottom of a 15 by 55 mm plastic Petri dish.

*Fertility assessment*: Egg fertility was determined by observing 8-d-old eggs under the dissecting microscope. Eggs with black dots (larval heads) were recorded as fertile.

*Sex identification at pupal stage*: Made according to Howell (1991).

### 3.2.3 Environmental Conditions

*Standard Conditions*: 20 ± 2°C, 16:8 L:D photoperiod (lights-on 0600 h and lights-off at 2200 h) (high frequency broad-spectrum biolux tubes, Osram, Germany) and 75 ± 10 % RH.

*Reverse-Light Conditions*: The same temperature, RH and photoperiod as above but lights-on at 1800 and lights-off at 1000h.

### 3.2.4 Definitions

*Fecundity* defined as the total number of eggs laid.

*Fertility* is the total number of fertile eggs laid

*Fertility rate* is the ratio of fertility vs fecundity.

### 3.2.5 Statistical Analysis and Reported Values

All statistical analyses were set at $P < 0.05$ and carried out using SAS STAT (SAS Institute 1996). Unless stated otherwise, all reported values are means ± SE.

## 3.3 Growth and Reproduction of *Cnephasia jactatana* Under Two Rearing Systems

### 3.3.1 Introduction

Before a research program can be established, it is essential to have a constant and reliable supply of insects. I initiated a research programme on the reproductive behaviour of *C. jactatana* and faced the decision of what rearing system to use to produce sufficient insects for experiments. There were essentially two systems to choose from: mass rearing (MR) and individual rearing (IR), the main differences are rearing scale and costs. For the IR system, obtaining insects of high quality is the priority and the cost is not a limiting factor while for the MR system, producing insects in high quantity at the lowest cost is the main purpose (Singh 1977). I needed to experimentally test the difference between the two rearing systems for *C. jactatana.*

An insect ideal for mass production should have three basic traits: a short life cycle, a high biotic potential and simple food requirements (Singh 1977). The main problems encountered in the MR are contamination of diets by microorganisms, diseases, and undesirable genetic or physiological changes such as loss of pheromone production or loss of ability to fly or to attack natural hosts (Singh 1977).

Ochieng'-Odero and Singh (1992) established a *C. jactatana* colony using an IR rearing method similar to that developed by Singh et al. (1985) for another tortricid species *E. postvittana.* There has been no report published on the comparison of biological parameters of *C. jactatana* reared by IR or MR systems. This section reports the findings on larval survival, pupal weight, emergence rate, fecundity and fertility under the MR and IR systems for *C. jactatana.*

### 3.3.2 Materials and Methods

Founder *C. jactatana* insects (100 pupae from generation 47) were obtained from the colony kept at HortResearch in Auckland. Pupae were maintained under standard conditions (Section 3.2.3) until adult emergence.

Newly emerged insects (< 12 h) were paired individually in test cylinders. Eggs were incubated and fertility assessed (Section 3.2.2). The following experiments were conducted under standard conditions (Section 3.2.3).

### 3.3.2.1 Rearing and Performance of Immature Stages

Two rearing systems were used as follows:

*Mass Rearing.* Five hundred neonate larvae (< 12 h old) were inoculated on to a 2-cm deep layer of general purpose diet (GPD) (Singh 1983) in each of eight test containers covered with paper sheets (Tyvec™, The Paper House Co. Auckland) and secured with a plastic ring, to allow air exchange. Each cylinder was considered as a replicate.

*Individual Rearing.* Neonate larvae (<12 h old) were individually inoculated onto 1 ml of GPD contained in 1080 plastic tubes (10 × 75mm, 4.5 ml). The tubes were then plugged with cotton wool and placed in test tube racks (cotton wool end upright). These tubes were divided into 6 groups of 180 tubes each as 6 replicates.

Pupae were harvested from cylinders and tubes after 6 weeks rearing, separated by sex, weighed and then kept individually in glass vials (Section 3.2.1), until adult emergence to ensure virginity.

The number of pupae resulting from different rearing systems was counted. Larval mortality rate was estimated as the percentage of larvae that did not reach pupal stage. Pupal mortality rate was recorded as the percentage that did not emerge. Adult emergence rate was calculated as the percentage of the larvae that developed to adults.

### 3.3.2.2 Fecundity and Fertility

Seventy-three pairs (<12 h old) from the MR system and 40 pairs (<12 h old) from the IR system were caged individually for their lifespan in test cylinders. Each pair was treated as a replicate. Eggs were incubated and fertility assessed (Section 3.2.2).

### 3.3.3 Statistical Analysis

Biological parameters between the two rearing systems were compared using a *t*-test. Percentage data were arcsine transformed and fecundity and fertility data were standardised before analysis (Steel et al. 1997). For fecundity and fertility, only those data from females that produced eggs were used for the analysis.

### 3.3.4 Results and Discussion

Insects in the IR system performed significantly better than those in the MR system (Table 3.1). The mean mortality rates of immature stages from the MR were significantly higher than those from the IR. About 74% of MR larvae died before pupation, which was about three times higher than IR ones. The pupal mortality rate from the MR was more than five times higher than from the IR. About 5% of MR insects reached adulthood while more than 60% of IR ones completed their development. Furthermore, both male and female pupae from the IR were significantly heavier than those from the MR.

The low performance of the insects in the MR system may have been caused by faeces and microbial contamination (Stone and Sims 1992). Also responsible for the high mortality may be overcrowding in the MR containers, which can lead to cannibalism between larvae or to pupae killed by active larvae (Singh 1977, Peters and Barbosa 1977). IR insects emerged within a period of 7 d while the emergence of MR insects was spread over 15 d, suggesting that developmental rate of MR insects was more variable.

**Table 3.1** Mean (± SE) survival rate and pupal weight of *Cnephasia jactatana* under mass rearing (MR) and individual rearing (IR) systems.

| Performance parameters | n | MR | n | IR | P |
|---|---|---|---|---|---|
| Larval mortality (%) | 4,000 | 74 ± 1.6 | 1,080 | 25 ± 3.9 | <0.001 |
| Pupal mortality (%) | 1,030 | 82 ± 2.6 | 813 | 15 ± 2.4 | <0.001 |
| Adult emergence (%) | 4,000 | 5 ± 0.8 | 1,080 | 64 ± 3.1 | <0.001 |
| Female pupal weight (mg) | 54 | 39 ± 1.0 | 32 | 45 ± 0.7 | 0.01 |
| Male pupal weight (mg) | 54 | 27 ± 0.7 | 32 | 31 ± 0.8 | 0.01 |

My observations suggested that the loss of water in the diet was another important factor responsible for the low performance of insects from the MR system. After 6 weeks of mass rearing, the diet dried out and became very hard, which made it difficult for larvae to feed and obtain sufficient water. This problem did not occur in the IR tubes in which diet was still soft and moist after 6 weeks of rearing. According to Singh et al. (1985), *E. postvittana* larvae required only 4 weeks to reach the pupal stage, suggesting that the problem of water stress in the mass rearing of this species may not be as serious as in the mass rearing of *C. jactatana*, which required two more weeks to complete its development.

Both fecundity and fertility in the IR system were significantly higher than in the MR system but the fertility rate of these two groups was similar (Table 3.2). It is suggested that the IR system provides much better conditions for the growth, development and reproduction of *C. jactatana*. Overcrowding and diet deterioration in the MR system may be the main cause in reduction of fecundity and fertility.

**Table 3.2** Mean (± SE) fecundity and fertility of *Cnephasia jactatana* under the Mass Rearing (n = 54) and Individual Rearing (n = 32) systems.

| Fecundity and fertility | MR | IR | P |
|---|---|---|---|
| No. of eggs/female | 273 ± 24 | 339 ± 29 | <0.05 |
| No. of fertile eggs/female | 198 ± 24 | 255 ± 29 | <0.05 |
| Fertility rate (%) | 60 ± 4.9 | 67 ± 5.4 | NS |

NS = non significant

Ochieng'-Odero and Singh (1992) reported that the fecundity of *C. jactatana* in an IR system varied from 70 to 200 eggs per female. However, in the present study, mean fecundity in the MR and IR systems, was 273 and 339 eggs per female respectively. Continuous selection of insects to laboratory rearing could be the reason for the difference.

## 3.4 Sexual Maturation and Adult Activity Patterns of *Cnephasia jactatana*

### 3.4.1 Introduction

Activities such as hatching, moulting, pupation and emergence in insects have a pronounced circadian rhythmicity (Saunders 1982). Selective advantages of the rhythmicity of these behaviours include the reduction of direct competition between species sharing the same resources and the synchronization of sexual activities in a population, increasing the efficiency of genetic isolation of sibling species (Saunders 1982). The study of circadian rhythms elucidates the spatial and temporal distributions of abundance of individuals, which is important for interpreting adult sampling estimates (Quiring 1994). Similarly, the study of adult activity patterns can enhance our ability to develop pest management tactics, such as mating disruption, or plant resistance based on non-preference (Quiring 1994).

Foster et al. (1993) identified the female sex pheromone for *C. jactatana*, and Ochieng'-Odero and Singh (1992) provided useful information on the biology of this species. However, there has been no report on its adult activity patterns, making the development of behaviour-based control tactics difficult. For example, it would be difficult to interpret adult trapping data without a good understanding of adult circadian and lifespan activity patterns. The aim of this section was to investigate adult emergence, sexual maturation, and daily and lifespan activity patterns.

### 3.4.2 Materials and Methods

#### 3.4.2.1 Emergence

Insects used in these experiments were mass-reared (Section 3.3.2.1). Pupae were separated by sex and kept in standard conditions. To determine the circadian rhythm of adult emergence, I recorded the emergence of 492 females and 523 males on an hourly basis.

### 3.4.2.2 Female Sexual Maturation

To better understand females reproductive activity patterns, I estimated their egg development status through dissecting. A total of 82 individually-reared virgin females (see Section 3.3.2.1) were killed by freezing (10 minutes in a domestic freezer, -20°C) when <12 h, 12-24 h and 36-48 h old. Their abdomens were removed and dissected in 1% saline solution under a dissecting microscope. The ovaries were separated out and immersed in 1% acetocarmine for 10 s before being transferred to clean saline solution. Immature eggs retained the stain, but the chorion of mature eggs prevented absorption (e.g. Fernando and Walter 1999). Unstained eggs were thus classed as mature and were presumed to be available for oviposition; stained eggs were classed as immature. All the mature eggs present in 4 out of 8 ovarioles were counted under the microscope.

### 3.4.2.3 Adult Activity Patterns

To observe adult activity patterns on a 24-h basis, I set up two bioassay rooms at standard and reverse-light conditions, respectively. Insects were mass-reared (Section 3.3.2.1). Recently harvested pupae were randomly split into two groups, one for each room. Adult activity patterns were studied by individually confining 65 pairs of newly emerged moths (< 12 h old) (31 in standard-light regime and 34 in reverse-light regime) in test cylinders.

As mating and courtship normally lasted more than 1 h, each pair was observed for 20 seconds/hour and their activities (feeding, courtship, mating or oviposition) were recorded. Feeding activity was scored when the insect touched the cotton wick with 10% sucrose solution with its proboscis. Courtship was recorded when the male jumped and fanned the wings over or around the female or if the male exposed his genitalia trying to engage the female's genitalia. Mating was scored if the two insects were engaged by the tip of the abdomen. Oviposition was recorded if a female was observed laying eggs or a new egg mass was found in the cylinder. Points on the graphs represent the total number of times an activity was recorded at a particular hour (Figure 3.3) or on a particular day (Figure 3.4). Observations during the dark period were made under two red photographic safe lamps (Phillips No. B22PF712B, EEC) (Webster and Cardé 1982).

### 3.4.3 Statistical Analysis

A Kolmogorov-Smirnov two-sample two-tailed test was used to determine whether developmental time differed between sexes (Siegel and Castelan 1988). The number of insects which had emerged per hour during the photophase (light period) and scotophase (dark period) was compared using an analysis of variance (ANOVA). The number of mature eggs in the ovaries was square root transformed (Steel et al. 1997) prior to analysis by *t*-test.

### 3.4.4 Results

#### 3.4.4.1 Emergence

Males emerged significantly earlier than females (KS test, $P < 0.01$) (Figure 3.1). On the first day of emergence, 7% of males and 4% of the females emerged. By the 3rd day after the first emergence occurred, more than 50% of males emerged while only 41% of females emerged. ANOVA analysis shows that during the photophase insects emerged at a rate of 0.73 ± 0.09 insects/h (mean ± SE), which was almost 50% higher than the emergence rate of 0.32 ± 0.07 insects/h during the scotophase ($P < 0.001$).

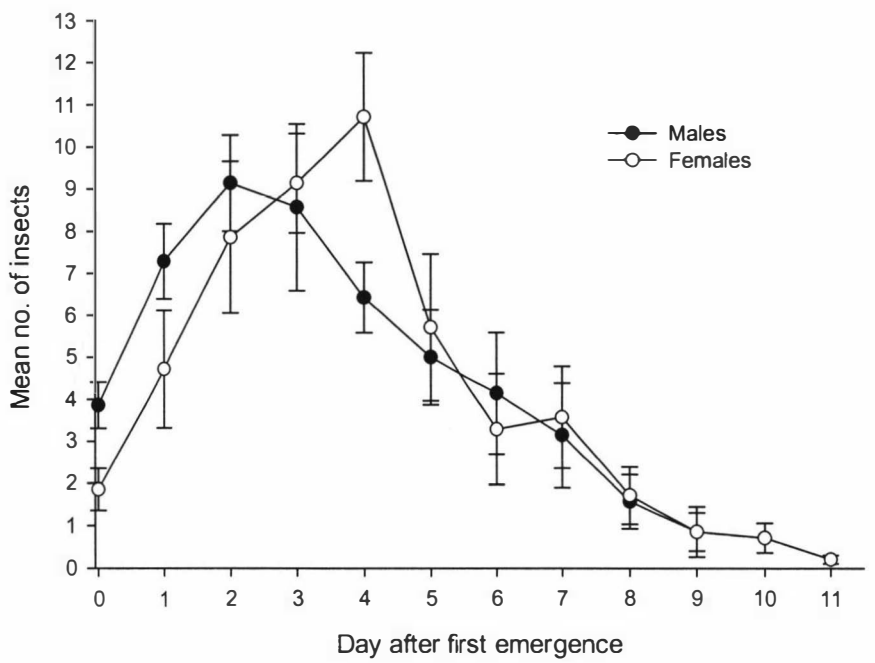


**Figure 3.1** Daily emergence of females and males of *Cnephasia jactatana* (n = 492 females and 523 males). Bars are SE.

### 3.4.4.2 Female Sexual Maturation

None of the 26 females dissected <12 h after emergence had any mature eggs. Significantly more mature eggs were present in 36-48 h old females than in younger females (Figure 3.2).

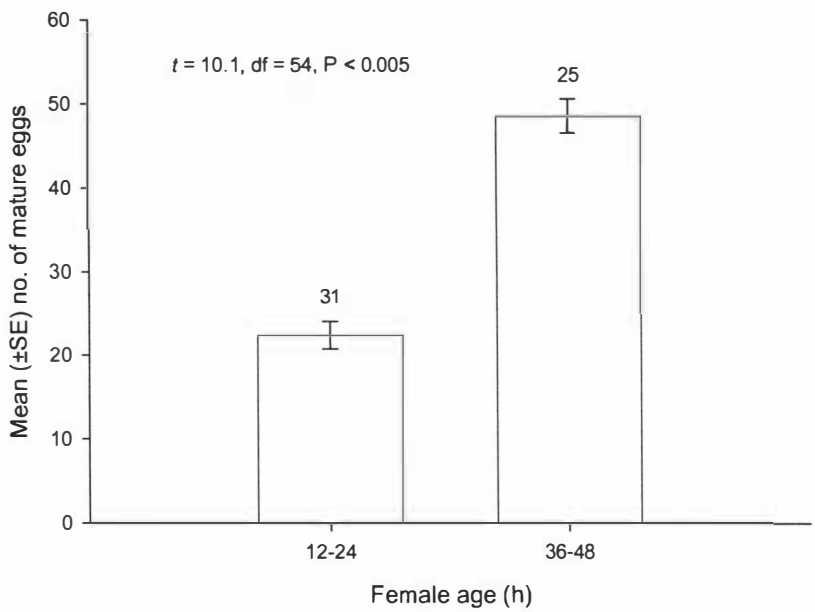


**Figure 3.2** Mean (± SE) number of mature eggs obtained from 4 ovarioles of females of different ages. Bars are SE. Sample sizes are shown on top of bars.

### 3.4.4.3 Adult Activity Patterns

Circadian rhythms of adult activities are summarized in Figure 3.3. Feeding occurred throughout the 24 h period, with a major peak at the end of the scotophase and two smaller peaks, one at 4 h into the scotophase and the other at the end of the photophase. Reproductive activity took place almost exclusively during the scotophase. Courtship activity peaked 3 and 6 h into the scotophase but was also observed outside of the scotophase during the final 4 h of the photophase. Mating was limited to the scotophase, peaking 4 and 6 h into the scotophase. Oviposition peaked 1 h into scotophase, and was rarely recorded during the photophase.

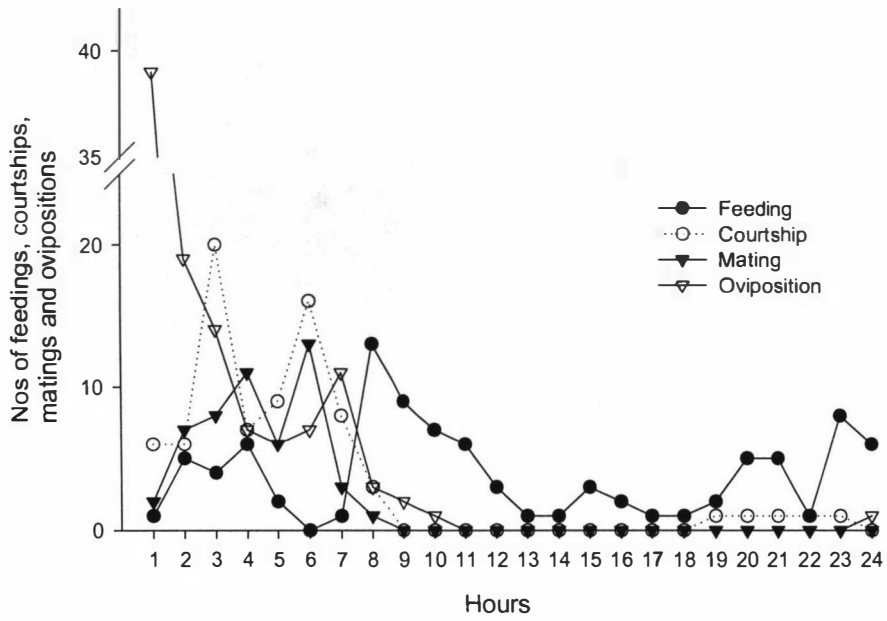


**Figure 3.3** Daily feeding and reproductive rhythms of *Cnephasia jactatana* (0-8 light off, 8-24 h light on).

Adult activity patterns were strongly influenced by age (Figure 3.4). Adults started feeding almost immediately after emergence, with a maximum feeding rate occurring 1 day after emergence. Courtship behaviour was first recorded 1 day after emergence, with most courtship displays occurring 2 days after emergence, followed by 2 small peaks 5 and 11 days after emergence. Mating activity reached a peak 2 days after emergence and then gradually declined with age. Females started to lay eggs 3 days following emergence, with most ovipositions occurring between 5 and 9 days after emergence. Only 15% of females laid eggs on the day when first mating occurred. However, more than 50% of females laid their first eggs 1 day after mating.

### 3.4.5 Discussion

*C. jactatana* females need significantly more time than males to complete their life cycle (Figure 3.1). Further, courtship display by males started at least 24 h earlier than mating, indicating that males become sexually active earlier than females (Figure 3.3 and Figure 3.4). Similar results were reported for the tortricid *Lobesia botrana* where 70% of males were able to mate 1 day after emergence but most

females were not receptive to mating attempts by males until 3 days after emergence (Torres-Vila et al. 1995).

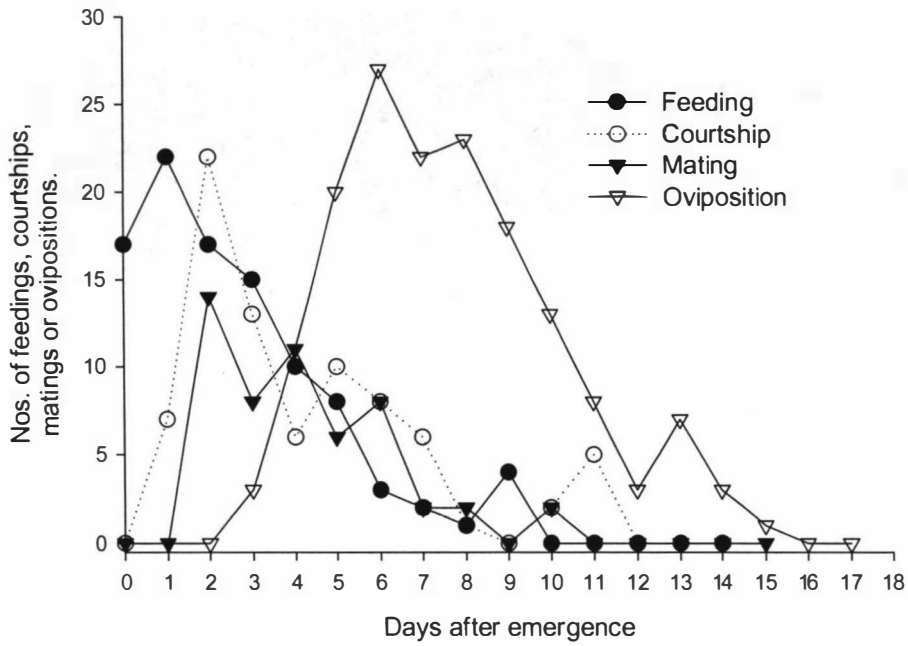


**Figure 3.4** Reproductive and feeding activities over the lifespan of *Cnephasia jactatana.*

Benz (1991) found that tortricid males possessed a fully formed reproductive system at the pupal stage while the newly emerged females had few or no mature eggs in their ovaries, requiring time to reach sexual maturity and rejecting early mating attempts. My results agree with those of Benz (1991). The number of mature eggs present in ovaries of 36-48-h-old *C. jactatana* females coincides with the number of eggs oviposited in the first egg mass (aprox. 80-100), suggesting that females accept mating only after they have sufficient mature eggs for their first oviposition. Studies of other Lepidoptera indicate that sexual activities are associated with the degree of ovarian development. For example, females of the armyworm *Mamestra configurata* did not start calling before the second scotophase when the first chorionated eggs appeared in their ovaries (Howlander and Gerber 1986). Similarly, unreceptive *Pseudaletia unipuncta* females had no chorionated eggs while receptive 3-d-old females possesed 120 mature eggs (Cusson and McNeil 1989).

Adult *C. jactatana* is a nocturnal insect and its sexual activities are closely related temporally (Figure 3.3). Each of the two large courtship peaks was closely followed by a mating peak, suggesting that courtship displays by males are very important for a successful mating. Species-specific circadian reproductive activity patterns have been reported in many insects. The pattern most similar to that of *C. jactatana* is found in *C. pumicana* (Chambon 1976), where mating occurs in the last 3-4 h of the scotophase and oviposition between dusk and 5 h into the scotophase. In the pyralid *Ectomyelois ceratoniae*, mating takes place between 5 and 6 h into the scotophase and oviposition is concentrated within first hour of the scotophase (Vetter et al. 1997). Cho and Boo (1998) reported that in the noctuid *Heliothis assulta*, oviposition occurs at the beginning of the scotophase, and mating occurs during the first half of the scotophase. However, in another noctuid species *Agrotis ypsilon* (Wang et al. 1983), oviposition mainly occurs 2 h after midnight whereas mating peaks just before dawn. Although activity patterns are variable, these examples have at least one thing in common, i.e. oviposition always took place earlier in the night than mating, suggesting that few females lay eggs on the same day as mating occurs. In *C. jactatana*, about 85% of females did not start to lay eggs until at least 1 day after mating. In *Cy. pomonella*, the movement of the sperm from the bursa copulatrix to vestibulum where fertilisation is achieved requires 4-5 hours (Benz 1991), reducing the time left during the same night, for oviposition. The short time between mating and the end of the scotophase may deter female oviposition behaviour until next scotophase. Egg laying is a long process and requires the female to be motionless. This increases the risk of predation, especially if the oviposition period needs to extend into the photophase.

## 3.5 Reproductive Potential and Population Increase of *Cnephasia jactatana*

### 3.5.1 Introduction

Ochieng'-Odero and Singh (1992) reported the general biology of *C. jactatana* but population growth parameters of this species were still not available prior to this study. These parameters can be used to improve rearing techniques, predict population dynamics, and develop pest management strategies. A well-documented method of assessing reproductive capacity and population growth in an insect species is the intrinsic rate of natural increase ($r_m$), which is defined as the rate of increase per female under specified physical conditions, in an unlimited environment where the effects of increasing density do not need to be considered (Birch 1948). This statistic combines, in a single value, information on survival and biotic potential of a species, reflecting the fecundity, longevity and speed of development of an insect. It allows comparisons of the population growth of an insect under different environment conditions, host plants or insect density, and thus may be useful in comparing reproductive strategies of different species (Andrewartha and Birch 1954).

The purpose of this experiment was to calculate the $r_m$ for *C. jactatana* to provide the foundation for the study of its reproductive fitness and behaviour in the following chapters.

### 3.5.2 Materials and Methods

#### 3.5.2.1 Data for Reproductive Potential and Population Increase

To estimate the reproductive capacity and population increase of mass-reared (Section 3.3.2.1) *C. jactatana* insects, I placed 65 breeding pairs of newly emerged moths (< 12h old) individually into test cylinders. Egg masses were incubated and fertility assessed as described in Section 3.2.2. Adult mortality was recorded daily.

Immature stages of *C. jactatana* took an average of 53 d from egg to emergence. Day 54 was thus considered to be the first day for adult *C. jactatana.*

I used the above data to generate adult fecundity and fertility tables covering the reproductive life of adult females, which was necessary for the study of rates of increase (Andrewartha and Birch 1954).

### 3.5.2.2 Estimation of Reproductive Potential and Population Increase

The computation of $r_m$ was based on the female population and estimated from the proportion of the original cohort surviving at age $x$ denoted as $l_x$, and the mean number of female offspring per unit of time produced by a female aged $x$ designated as $m_x$ (Birch 1948, Carey 1993).

Andrewartha and Birch (1954) described two methods of calculating $r_m$, the approximate, and the accurate. Laughlin (1965) proposed the approximate statistic for the capacity of increase ($r_c$):

$$r_c = \frac{\log_e Ro}{T_c} \qquad \text{A}$$

where $Ro$ = net reproductive rate or $\sum l_x m_x$; $T_c$ = cohort generation time [mean age of the mothers in a cohort at the birth of female offspring (oviposition)] or $\frac{\sum x l_x m_x}{\sum l_x m_x}$.

According to Birch (1948), the accurate value of $r_m$ for any population is the one that satisfies the formula:

$$\sum e^{7-r_m x} l_x m_x = 1097 \qquad \text{B}$$

The first approximation of $r_c$ obtained from equation A was substituted in equation B. After two iterations of equation B computed with trial values (e.g. Singh and Singh 1994) of $r_c$ (a slightly larger or lower value), a straight line was fitted between the two values and analytical computation of $r_m$ was obtained. A jackknife technique (Miller 1974, Meyer et al. 1986) was applied to estimate the variability of $r_m$, $Ro$ and $T_c$ (e.g. Sánchez et al. 1997).

Other population parameters computed included the finite rate of increase ($\lambda$, defined as the number of times the population multiplies in a unit of time or $\lambda$ = antilog $e^{r_m}$), and the doubling time ($DT$, defined as the time required for the

population to double or $DT = \frac{\log_e 2}{r_m}$). A detailed explanation of these parameters and their computation was given by Birch (1948) and Carey (1993).

### 3.5.3 Results

In general, the first eggs were laid 3 d after emergence; after which time daily fecundity increased rapidly from 2.86 females/female/d to the maximum of 18.14 females/female/d on the 6th d, and then declined towards zero on the 15th d (Figure 3.5).

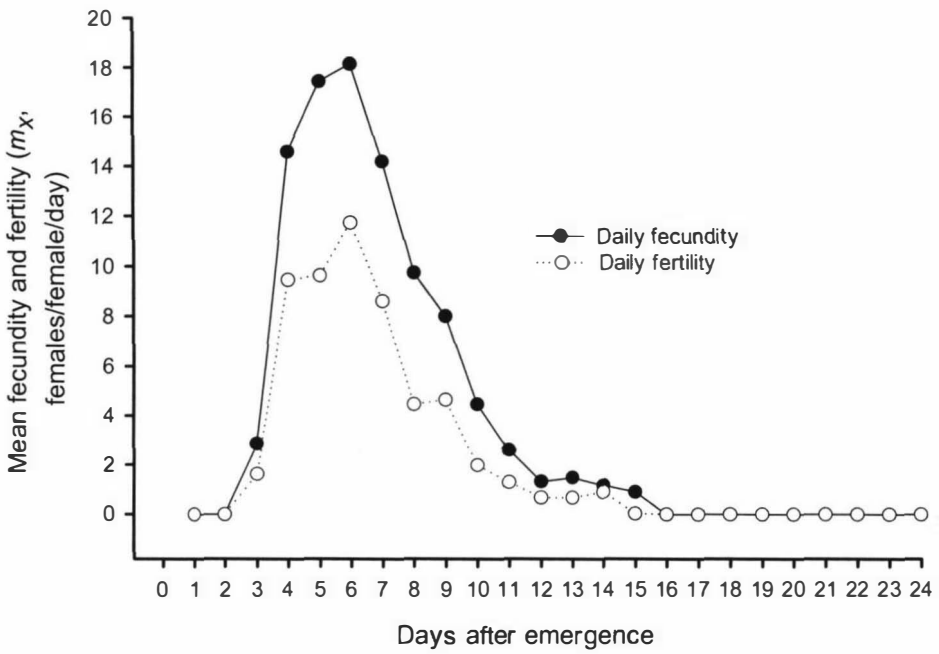


**Figure 3.5** Daily fecundity and fertility for *Cnephasia jactatana*.

Daily fertility rate ranged from 45 to 65% between 3 and 13 d after emergence (Figure 3.6). It sharply increased up to 80% 14 d after emergence, and then quickly decreased to the minimum. The sharp increase, however, may not be realistic because of the low number of eggs laid 11 d after emergence. Figure 3.6 also

shows that about 50% of the total fertilised eggs were laid between 5 and 6 d after emergence.

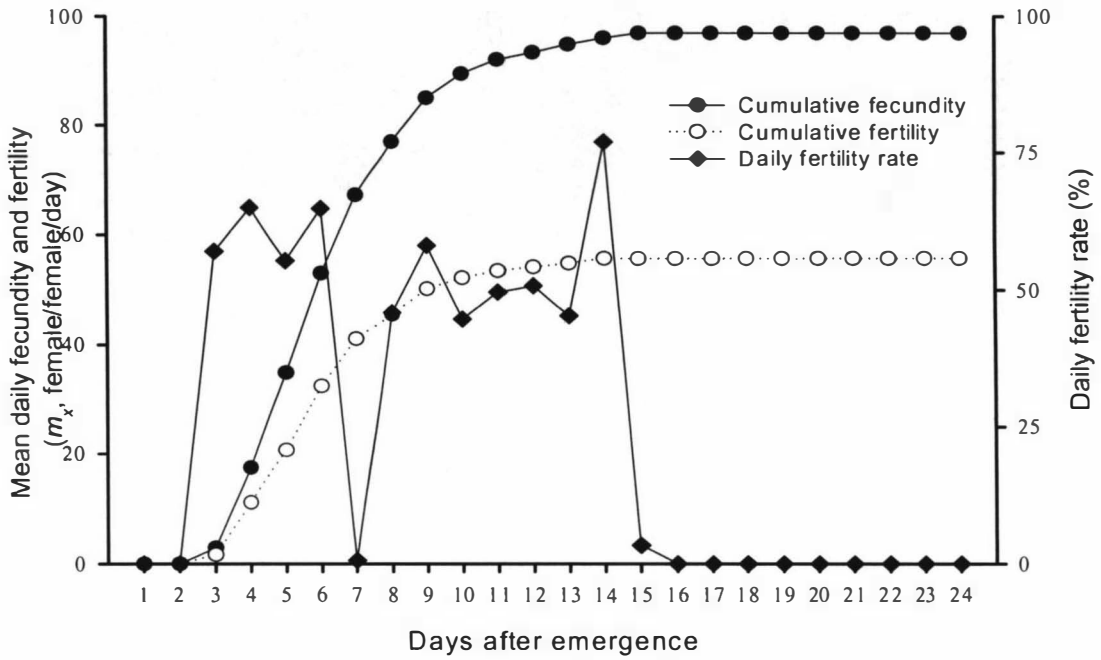


**Figure 3.6** Daily cumulative fecundity, fertility and daily fertility rate of *Cnephasia jactatana.*

Adults attained the greatest mean daily fecundity ($m_x$ = 18.14 females/female/d) and fertility ($m_x$ = 11.74 females/female/d) 59 d after egg hatch, which subsequently declined on day 69 (Figure 3.5). The mean overall fecundity and fertility were 189.75 and 109.8 eggs/female, respectively (Table 3.3).

The generation time ($T_c$) obtained from the daily observation of egg laying and survival rate ($l_x$) was 59.45 d for fecundity and 59.3 d for fertility. The net reproductive rate (*Ro*) was 91.75 females/female and 53.3 females/female for fecundity and fertility, respectively. Therefore, according to Birch (1948), a population of *C. jactatana* would multiply 91.75 or 53.3 times in each generation. An increase of the population by 53.3 times per generation should be more realistic, since only fertilised eggs contribute to the next generation (Table 3.3).

**Table 3.3** Population parameters for fecundity and fertility of *Cnephasia jactatana*

| Population growth statistics | Calculated value | |
|---|---|---|
| | Fecundity | Fertility |
| Reproduction | 189.75 ± 23.3 egg/female<br>4.03 ± 1.24 egg/female/d<br>50% egg oviposited at 59$^{th}$ d | 109.8 ± 21.07 egg/female<br>2.32 ± 0.75 egg/female/d<br>50% egg oviposited at 59$^{th}$ d. |
| Net reproductive rate Ro | 91.75 ± 0.2088 females/female | 53.31 ± 0.1935 females/ female |
| Mean length of generation $T_c$ | 59.45 d | 59.3 d |
| Capacity for increase $r_c$ | 0.076 females/female/d | 0.067 females/female/d |
| Trial values of $r_c$ | 0.05 and 0.09 females/female/d | 0.04 and 0.08 females/ female/d |
| Intrinsic rate of natural increase $r_m$ | 0.0847 ± 0.00003 females/ female/d | 0.0839 ± 0.00005 females/ female/d |
| Corrected generation time T | 53.35 ± 0.0063 d | 47.39 ± 0.0095 d |
| Finite rate of increase $\lambda$ | 1.08 females/female/d | 1.08 females/female/d |
| Doubling time DT | 8.18 d | 8.26 d |
| Gross reproduction rate $GRR = \sum m_x$ | 96.92 females/female/generation | 55.78 females/female/generation |
| Weekly multiplication of population $\lambda^7$ | 1.80 | 1.79 |
| Hypothetical $F_2$ female $Ro^2$ | 8418 | 2841 |

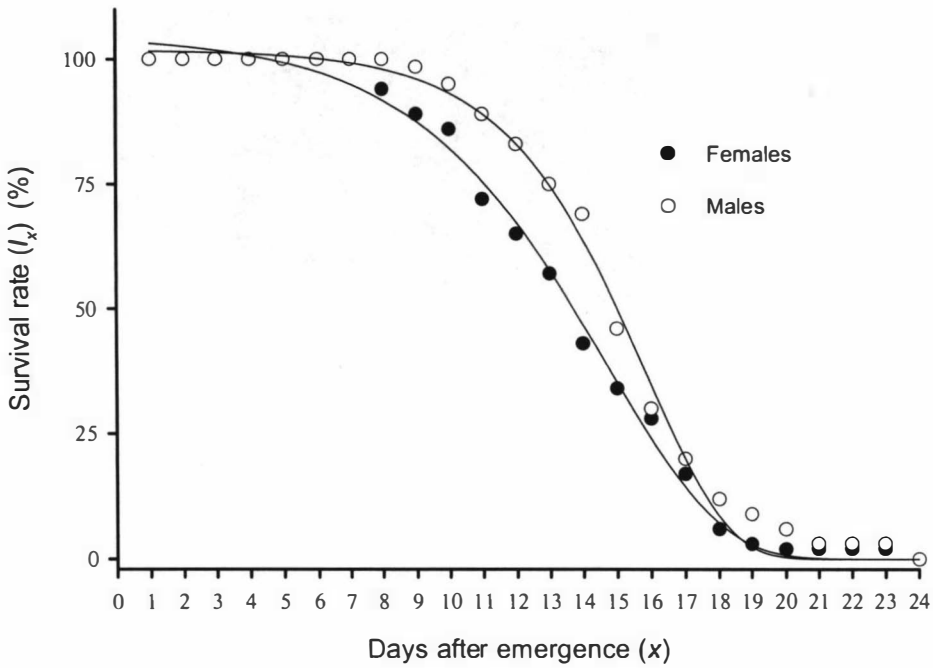


**Figure 3.7** Survival rate of adult female and male of *Cnephasia jactatana*.

The capacity for increase ($r_c$) for fecundity and fertility was 0.076 and 0.067 females/female/d, respectively, but the intrinsic rate of natural increase ($r_m$) was 0.0847 and 0.0839 females/female/d, respectively. From these values, a finite rate of increase ($\lambda$) was determined as 1.08 females (Table 3.3). This value represents a constant by which the population is expected to multiply each day.

The population doubled once in 8.18 and 8.26 d, respectively. If one assumes that all the births are concentrated at one moment, two consecutive generations would be separated by 53.35 and 47.39 d (computed using $r_m$), respectively, and the population of *C. jactatana* would increase 1.8 and 1.79 times in 7 d, respectively. Population parameters for fecundity and fertility of *C. jactatana* and the standard errors generated through jackknife technique for $r_m$, *Ro* and *T* are presented in Table 3.3.

Days 8 and 9 after emergence marked the first female and male casualties, respectively. More than 50% of males and females lived for 13 d and 25% lived for

at least 16 d (Figure 3.7). It is also indicated that most males lived longer than females.

### 3.5.4 Discussion

The economic importance of *C. jactatana* to the kiwifruit industry depends on the quantitative components of its reproductive potential and other attributes such as the number of generations on the crop and the insect population density. High fecundity, fertility and survival within the first 8 d of the adult life are the principal components of the intrinsic rate of natural increase ($r_m$).

The growth of the *C. jactatana* population was determined by the age of the female at the commencement of oviposition and the intensity of oviposition during the first few days of the oviposition period. In general terms, *C. jactatana* presented an early reproduction period with females having laid more than 50% of their total eggs by the time they were 6 d old. By concentrating the reproductive effort in the early days of its adult stage, *C. jactatana* maximises its ability to maintain its population, as the impact of predators on the older females has little influence on population growth (Price 1997). As Birch (1948) pointed out, the earlier the oviposition effort is, the greater its contribution to the $r_m$ value will be. For example, the first 5 d after emergence accounts for almost 65 % of the $r_m$ value for *C. jactatana*. However, late ovipositions may also play a role in maintaining the population in unfavourable conditions like pesticide applications or climatic changes (Kennedy et al. 1996).

The intrinsic rate of natural increase varies between species. For example, $r_m$ estimated for *C. jactatana* in this study ($r_m$ = 0.0847), was higher than that for the tortricid *Epinotia aporema* ($r_m$ = 0.0570) (Sánchez et al. 1997) but lower than that for the arctiid *Spilosoma obliqua* ($r_m$ = 0.1295) (Varatharajan et al. 1998) and the noctuid *Plusia eriosoma* ($r_m$ = 0.1701) (Tripathi and Shahi 1992). A high $r_m$ may mean that under natural conditions the population suffers a high mortality and a low $r_m$ may indicate low mortality in nature and would lead to search for adaptive methods for avoiding mortality (Price 1997).

When the moth age distribution is stable and in an unlimited environment, $r_m$ can be used to predict the population growth of *C. jactatana* using the model:

$$N_t = N_o e^{r_m t}$$

where $N_t$ is the predicted moth density at time $t$ (days), $N_o$ is the initial population density, and $r_m$ is the intrinsic rate of natural increase (Carey 1993). This model establishes a foundation for examining population growth patterns over short periods and provides the initial framework from which to build more complicated population growth models. Those more complicated models simulates competition between species or the predator-prey interaction, and contribute to our understanding of pest population dynamics, especially population regulation by biotic and abiotic factors (Carey 1993, Schowalter 2000).

The use of $r_c$ instead of $r_m$ is acceptable when distinct generations are present as $r_c$ computation is simpler and both figures are similar. However, when overlapping generations occur, $r_m$ is larger than $r_c$. Therefore, the use of $r_c$ in this situation will underestimate population growth of the species (Southwood and Henderson 2000).

This study established the foundation for estimation of *C. jactatana* population increase, from which further modelling in different environments can be built up. The development and commercial use of new kiwifruit varieties and the establishment of cultural innovations can modify the availability and quality of food accessible for *C. jactatana* and thus the environmental conditions. Different varieties and cultural environments may produce different $r_m$ values (e.g. Leather and Dixon 1982). Determining the reproductive potential and intrinsic rate of natural increase of *C. jactatana* for each new situation can provide information to assist in evaluating new varieties of kiwifruit and cultural innovations in their cultivation.

## 3.6 Effect of Bodyweight on Reproductive Fitness in *Cnephasia jactatana*

### 3.6.1 Introduction

The fitness consequences of size/weight and its correlates, especially the supply of sperm or eggs and adult longevity, are important in population dynamics and essential for understanding and modelling life story evolution and behavioural decisions regarding host choice, egg clutch size and sex ratio (Tammaru 1998, Cloutier et al. 2000). Size-fitness relationships are also relevant to mass rearing programs as weight is commonly monitored in the laboratory insect populations and is regularly incorporated into process and product analysis of production systems as a measure of quality (Chambers and Ashley 1984). Knowledge of the relationship between bodyweight and reproductive fitness is important for the development of insect control techniques. For example, the implementation of the sterile insect technique (SIT) relies heavily on a constant supply of insects with desirable characteristics such as optimal bodyweight. The study of weight-fitness relationships helps clarify the sexual selection and mating system of the species.

The aim of this section was to understand the relationship between body weight and reproductive fitness in *C. jactatana* by studying how and to what extent body weight affected fecundity and fertility, and whether it affected both sexes in a similar way.

### 3.6.2 Materials and Methods

The insects were obtained as pupae from the colony kept at HortReseach, Auckland. Pupae inside the rearing tubes (75 mm in length by 10 mm diameter), were send by courier overnight and arrived the next day at the Massey University Entomology and IPM Laboratory. Pupae were separated by sex and weighed as in Section 3.2.1. All experiments were conducted under standard conditions (Section 3.2.3).

The effect of pupal weight on fecundity and fertility was studied by confining 175 breeding pairs of newly emerged moths (< 24 h old) individually for the duration of their lifespan in test cylinders. A complete factorial block design was used for this

experiment, where each sex (factor) had three different pupal weights: light, average and heavy. A light or heavy pupa was defined as the one whose weight went below or above one standard deviation of the population. The experimental design produced nine treatments (3 female weights × 3 male weights) of breeding pairs (Table 3.4). Egg masses collected daily were treated as described in Section 3.2.2.

**Table 3.4** Number of *Cnephasia jactatana* breeding pairs in different bodyweight combinations (n = 175 pairs)

| Female class | Male class | n |
|---|---|---|
| Light | Light | 17 |
| Light | Average | 18 |
| Light | Heavy | 18 |
| Average | Light | 22 |
| Average | Average | 24 |
| Average | Heavy | 21 |
| Heavy | Light | 14 |
| Heavy | Average | 24 |
| Heavy | Heavy | 16 |

To determine whether pupal weight had any effect on daily fecundity and fertility, preoviposition, oviposition and postoviposition periods, I recorded the first and last day females laid eggs as well as the number of total and fertile eggs laid daily by each female.

### 3.6.3 Statistical Analysis

A *t*-test was used to compare body weight between sexes. Regression analysis and 2-way analysis of variance (ANOVA) followed by a least significant difference test (LSD) were used to analyse the influence of the pupal weight on fecundity, fertility, fertility rate, daily fecundity and fertility, preoviposition, oviposition and postoviposition periods. Fertility rate was arcsine transformed prior to analysis (Steel et al. 1997).

### 3.6.4 Results

Mean female pupal weight (41.54 ± 0.59 mg) was significantly greater than male pupal weight (28.0 ± 0.33 mg) (*t*-test, $t = 20$, df = 112, $P < 0.001$). Females that mated with males under 11.5 mg failed to lay any fertile eggs. No minimal egg-laying pupal weight threshold was detected for females.

Male effect and the interaction male-female were not significant (P>0.05) in all cases. Heavy and average weight females laid significantly more eggs than light females (Table 3.5). Fertility data revealed that heavy females laid significantly more fertile eggs than light females, with average weight females ovipositing an intermediate number of fertile eggs (Table 3.5).

**Table 3.5** Reproductive fitness parameters (mean ± SE) of *Cnephasia jactatana* females of different bodyweight

| Parameter | Female weight class | | | F | P |
|---|---|---|---|---|---|
| | Light | Average | Heavy | | |
| Lifetime fecundity | 518 ± 26b | 595 ± 23a | 648 ± 25a | 5.52 | 0.004 |
| Lifetime fertility | 438 ± 28b | 493 ± 26ab | 562 ± 31a | 3.14 | 0.045 |
| Fecundity of the first egg clutch | 67.2 ± 3.94b | 87.89 ± 3.75a | 84.9 ± 4.42a | 7.59 | 0.0007 |
| Fertility of the first egg clutch | 58.14 ± 4.71b | 75.62 ± 4.10a | 75.1 ± 5.32a | 4.4 | 0.013 |
| Daily fecundity | 39.12 ± 1.73b | 44.80 ± 1.85a | 48.36 ± 1.99a | 7.17 | 0.001 |
| Daily fertility | 32.86 ± 2.06b | 36.79 ± 1.74ab | 40.35 ± 2.41a | 4.01 | 0.020 |
| Preoviposition period (d) | 3.96 ± 0.12b | 3.68 ± 0.08ab | 3.57 ± 0.11a | 3.08 | 0.048 |

Means followed by the same letter in row are not significantly different (ANOVA followed by LSD, P > 0.05)

The mean fertility rate obtained for the whole experiment was 82.33 ± 0.17% and it was not affected by the parents' pupal weight ($F = 0.24$; $df = 13,163$; $P > 0.05$).

The fecundity and fertility of heavy and average females in the first egg clutch were significantly greater than those of light females (Table 3.5). However, female pupal weight did not affect the fertility rate of the first egg clutch, which ranged from 80% to 89% ($F = 0.44$; $df = 2,163$; $P > 0.05$).

For light and average weight females, both fecundity and fertility increased linearly as females' pupal weight increased (Figure 3.8 A–B and Figure 3.9 A-B). However, in heavy females fecundity and fertility did not increase or even slightly decreased with the increase of body weight (Figure 3.8 C and Figure 3.9 C).

Male pupal weight showed a positive linear relationship with both fecundity (Figure 3.10 A-C) and fertility (Figure 4.11 A-C) for all weight groups.

### 3.6.5 Discussion

Sexual selection assumes that one or more components of fitness are a function of increasing body size (Roff 1992, Andersson 1994) through an allometric or linear relationship (Klingenberg and Spence 1977). Nevertheless, organisms do not increase in size continuously (Roff 1981, Blanckenhorn 2000, Thompson & Fincke 2002), and there is a limited (e.g. Blanckenhorn et al. 1999) or an optimal size (Roff 1981). This so-called "stabilizing sexual selection" predicts that heavier individuals do not always have a better reproductive fitness than average and/or light organisms of the same species (Thompson and Fincke 2002). Furthermore, some authors have demonstrated the selection for small males (Petrie 1983, Andersson 1994, Fox et al. 1995, Beeler et al. 2002), size-assortive selection (Arnqvist et al. 1996, Breuker and Brafefield 2002, Peckarsky et al. 2002) or asymptote fecundity at large body sizes (Blanckenhorn et al. 1999). An example of the latter is the pyralid *Parapediasia teterrella*. In this species reproductive effort is positively associated with the female body weight at or below the average but negatively associated with the weight exceeding the average of the population (Marshall 1990). My results show light females presented reduced fecundity and fertility when compared to average

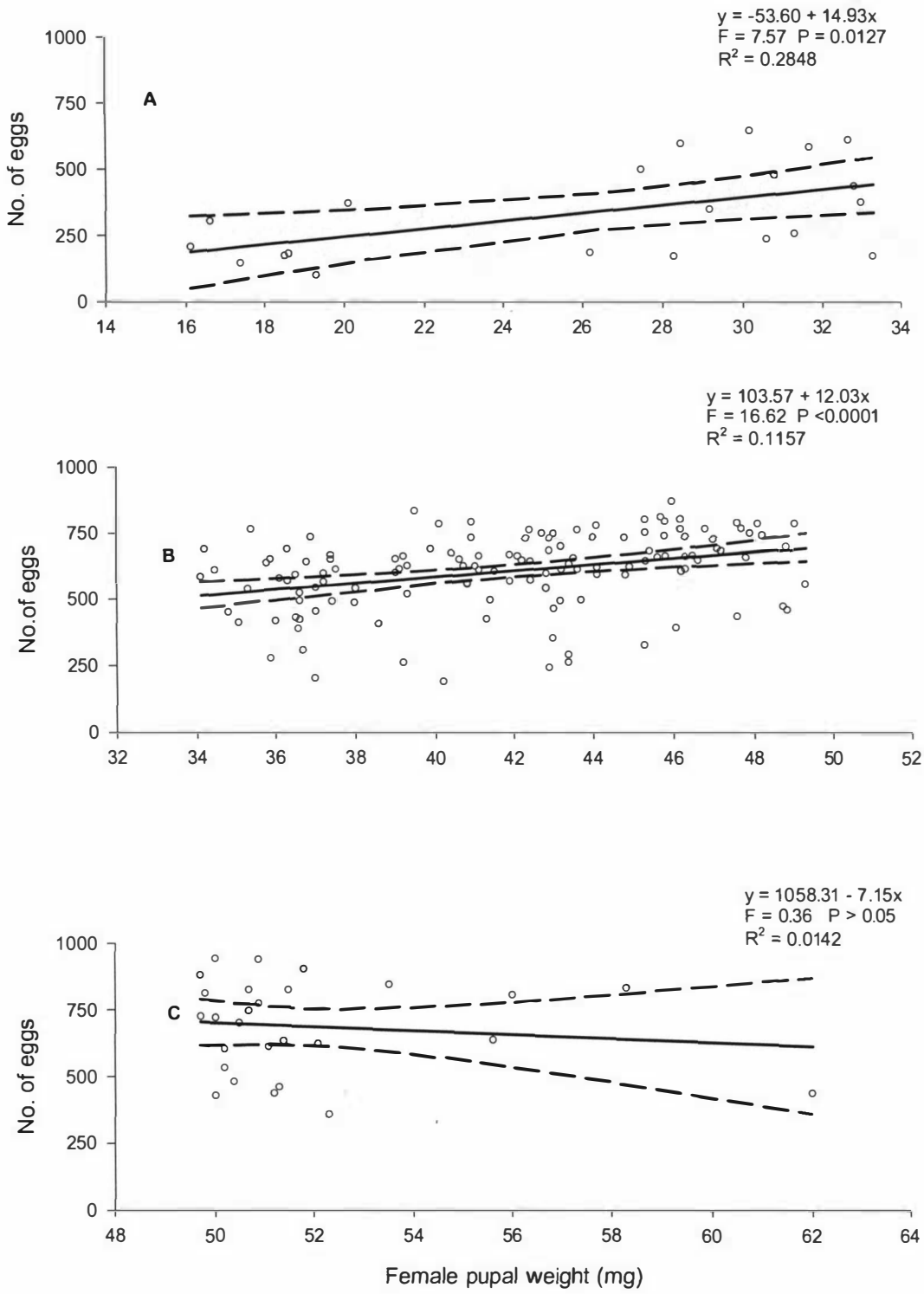


**Figure 3.8** Relationship between female pupal weight and fecundity. A, light females; B, average females, and C, heavy females. Lines represent predicted values at 95 % confident interval (broken lines).

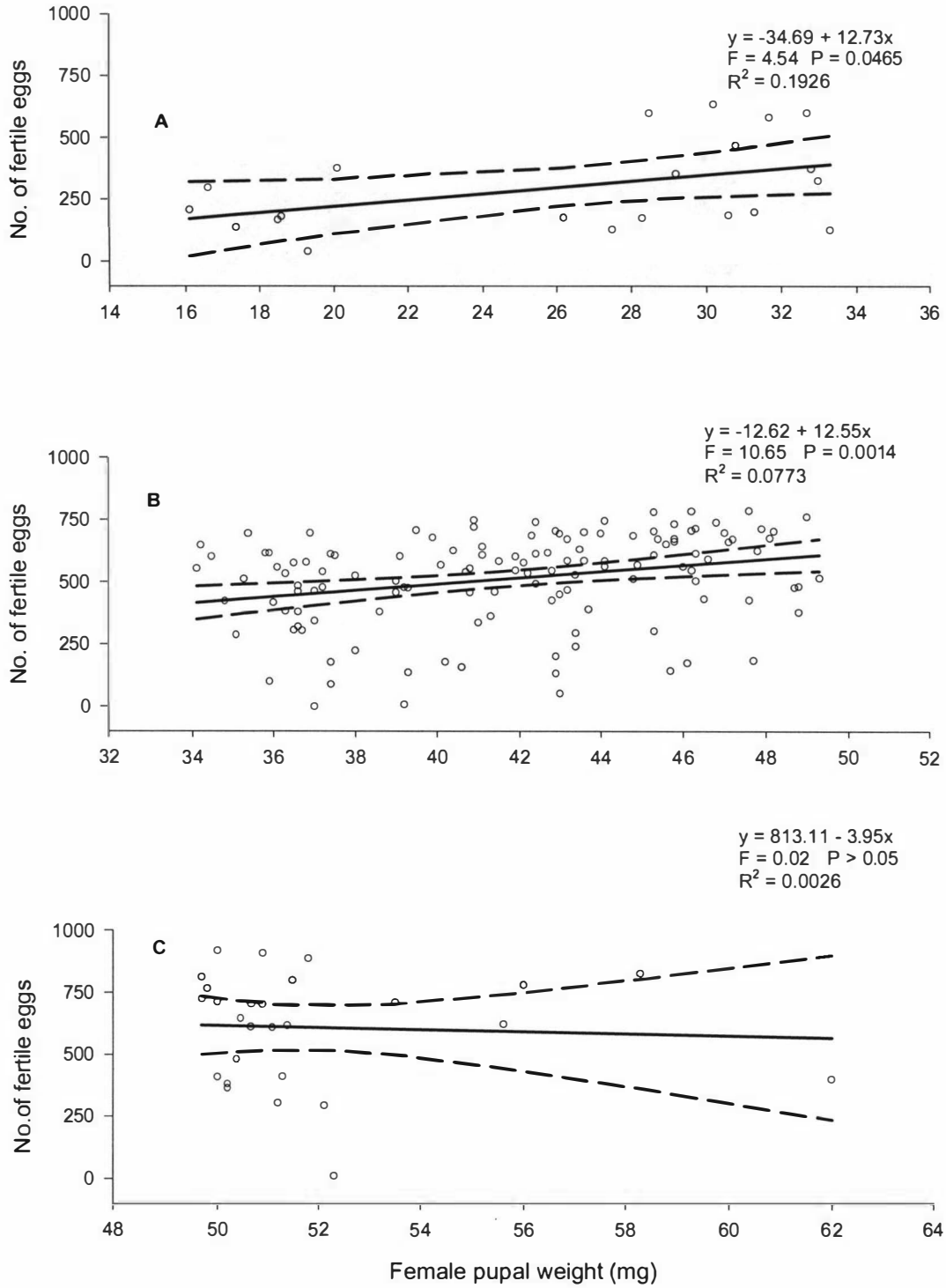


**Figure 3.9** Relationship between female pupal weight and fertility. A, light females; B, average females, and C, heavy females. Lines represent predicted values at 95 % confident interval (broken lines).

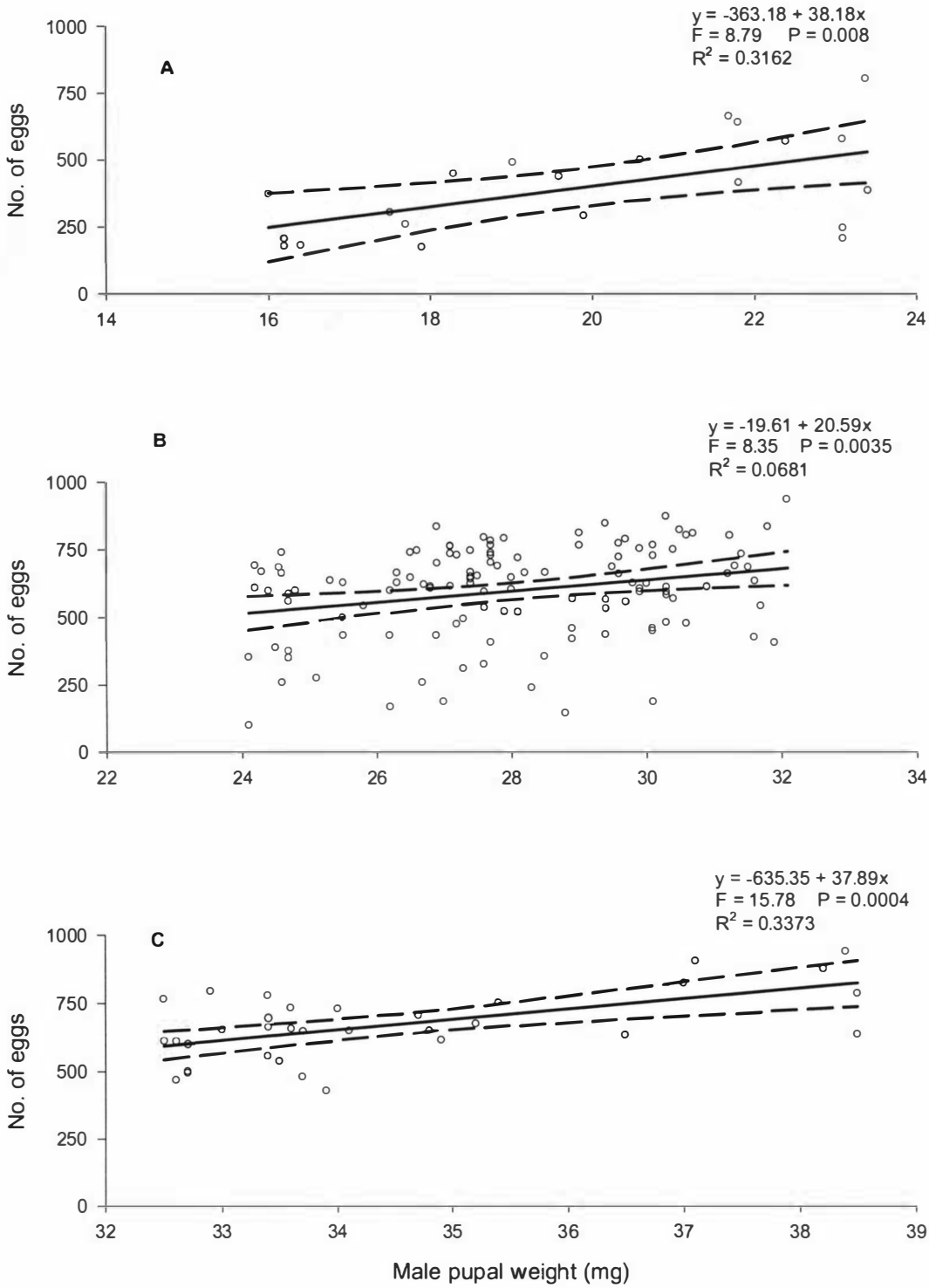


**Figure 3.10** Relationship between male pupal weight and fecundity. A, light males; B, average males, and C, heavy males. Lines represent predicted values at 95 % confident interval (broken lines).

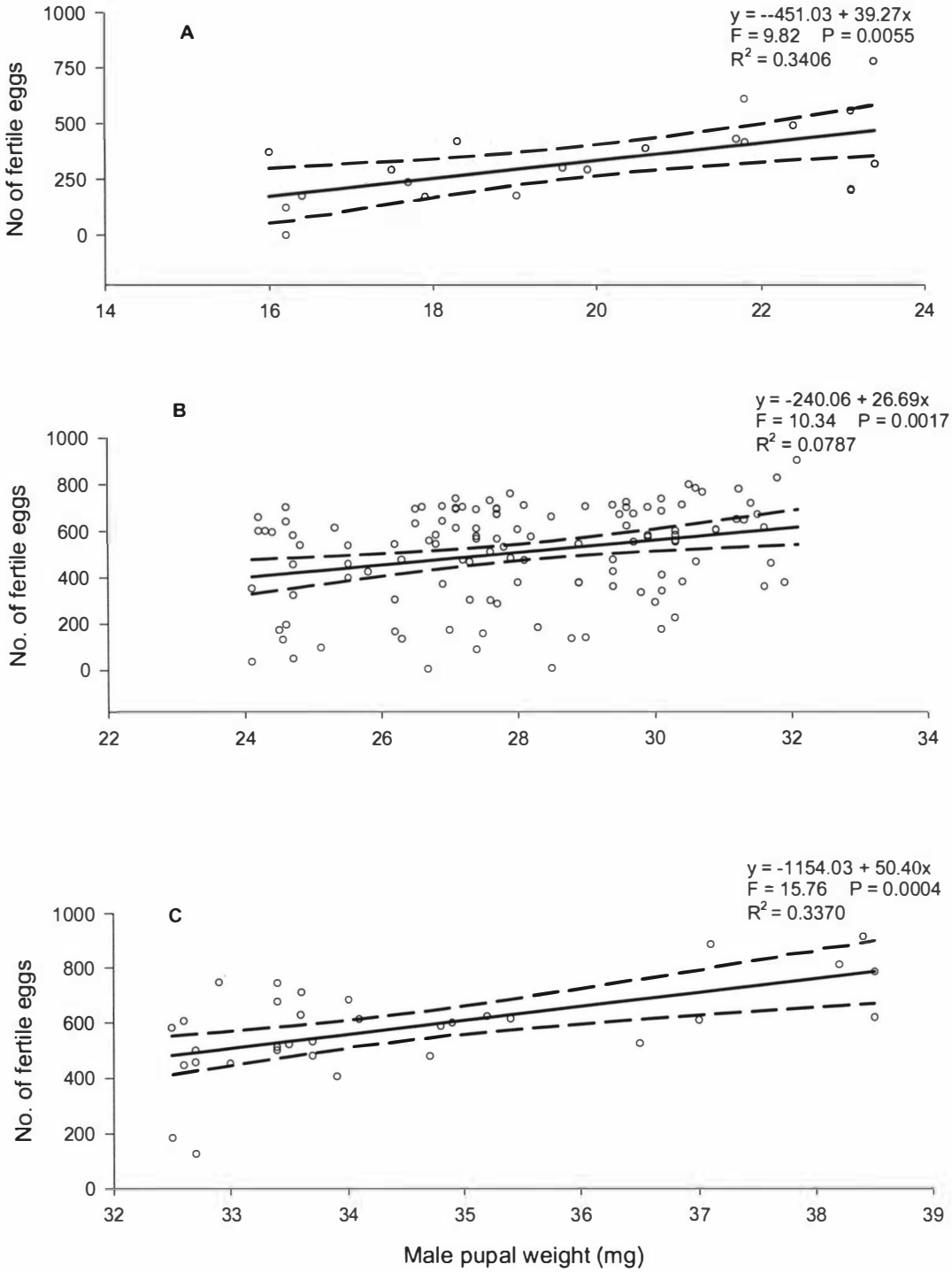


**Figure 3.11** Relationship between male pupal weight and fertility. A, light males; B, average males, and C, heavy males. Lines represent predicted values at 95 % confident interval (broken lines).

and heavy individuals. However, as Figures 3.8 A-B and 3.9 A-B show, a linear pattern occurred when pupal weight was light or average, but no further reproductive increase was observed within heavy females (Figures 3.8 C and 3.9 C), suggesting an upper limit for the beneficial effect of bodyweight on female reproductive output.

For *C. jactatana* females, my results support an optimal size instead of a the-larger-the-better approach as light females give a reduced reproductive output and obese females do not have increased fitness gain. It is suggested that females at the weight extremes (very light or very heavy) may present developmental and physiological handicaps. Roff (1981) and Tanaka (1981) state that it may be advantageous for an insect to attain a certain standard (optimal) size when it reaches the adult stage. An increase in potential fecundity above a certain level may increase the risk of female mortality (Nalepa and Mullins 1992) or predation risk (Blanckenhorn 2000). However, for *C. jactatana* males, the-larger-the-better strategy appears to be dominant (Figures 3.10 and 3.11) with a minimal pupal weight threshold for fertilisation. In order to produce a "functional adult", pupae must achieve a critical weight ($P_{cw}$), which was determined to be 18 and 11.6 mg for *C. jactatana* females and males, respectively (Ochieng'-Odero 1990). My results agree to the proposed $P_{cw}$ for males, but females as light as 16.1 mg produced eggs, lowering the proposed $P_{cw}$.

Heavy and average *C. jactatana* females laid eggs significantly earlier than light ones in this study. According to Birch (1948) and Carey (1993), females that oviposit earlier make a greater contribution to the growth of the population, which has implications for pest management. In many other species, heavy females have larger (Marshall 1990, Iyengar and Eisner 2002) and more eggs (Cloutier et al. 2000, García-Barros 2000) ready for oviposition and start to oviposit before other females. Therefore, males that mate with heavy females may sire more offspring, which have higher survival probabilities (Iyengar and Eisner 2002). The offspring face less competition for food and shelter, i.e. more suitable oviposition sites are available for the female in early stage of the population growth, increasing both female and male fitness.

In a mass-production facility, weight is an important parameter when we evaluate the quality of the laboratory populations (Chambers and Ashley 1984).

Production and release of heavy sterile males to the wild have proven to produce better control, because light or average sized sterile insects achieve less matings than wild insects (Orozco and López 1993). My results suggest that the development of any control tactic that could selectively remove heavy and average *C. jactatana* insects from the field would further reduce population growth.

# CHAPTER 4
# EFFECT OF MATING DELAY AND REMATING ON REPRODUCTIVE FITNESS OF *CNEPHASIA JACTATANA*

## 4.1 General Introduction

Reproductive fitness correlates highly with mating behaviour. According to Darwin's sexual selection theory, male reproductive fitness is limited by the number of ova he fertilises. Thus, male reproductive fitness increases with number of matings. On the contrary, female reproductive fitness is limited by sperm supply and her egg-load. However, male and female reproductive fitness is highly dependent on age at mating since only a short period separates gonadic maturation from gonadic senescence. This chapter reports the impact of mating delay and remating behaviour on the reproductive fitness of *Cnephasia jactatana.*

## 4.2 Effect of Mating Delay on Reproductive Fitness of *Cnephasia jactatana*

### 4.2.1 Introduction

Insect reproduction involves two behaviours: mating and oviposition. These behaviours must occur within a limited period because the physiology of both sexes changes over time. Therefore, the age of the insects when they mate influences their reproductive fitness and population growth.

Various reports show that the delay in mating generally shortens the oviposition period and reduces fecundity and fertility. This reduction in fertility and fecundity may be the result of oviposition of unfertilised eggs by virgin females, affecting their overall fecundity (Foster et al. 1995); the lack of attractiveness of old females to potential mates, reducing their mating opportunities (Lingren et al. 1988, Spurgeon et al. 1995, Proshold 1996), or the reception of sperm of low quality and

quantity from old males (Unnithan and Paye 1991, Rogers and Marti 1994, Vickers 1997).

It is essential to understand the mating process, the factors controlling it, and its effect on reproductive potential, if pheromonal control strategies aimed at interfering with a pest's reproduction are to be developed (Unnithan and Paye 1991). A control technique that restricts the availability of males (e.g. mating disruption) influences a pest population by preventing mating in some females and by delaying it in others. Both situations can suppress the pest population (Ellis and Steele 1982, Vickers 1997). However, whether or not the disruption tactic is successful in the control of an insect pest largely depends on our understanding of the reproductive behaviour of the pest (Cardé and Minks 1995).

Mating disruption in commercial crops has proven to be successful for the control of *Epiphyas postvittana* (Shaw et al. 1993), *Lobesia botrana* (Cardé and Minks 1995) and *Cydia pomonella* (Calkins 1998, Angeli et al. 1999), but not effective for other tortricids such as *Grapholita molesta* where preventing mating is mandatory (Fraser and Trimble 2001). Such variation is probably due to the difference in mating systems between species.

Foster et al. (1993) identified the female sex pheromone of *C. jactatana*. More recently, Stevens and McKenna (1999) suggested that mating disruption could achieve a better control of the late-season infestation. This Section examines whether and to what extent the delay in mating affected the reproductive fitness of *C. jactatana*, providing information vital to assessing the feasibility of pheromone-based control methods.

### 4.2.2 Materials and Methods

#### 4.2.2.1 Insects

Insects were individually reared (Section 3.3.2.1), and separated by sex and pupae weighed according to Section 3.2.2 Newly emerged females and males were kept individually in glass vials and placed in separate rooms until the experimental day. Standard conditions (Section 3.2.3) were used for all the experiments.

#### 4.2.2.2 Effect of Mating Delay on Reproductive Fitness and Spermatophore Size

According to Section 3.4 most *C. jactatana* females were not sexually mature until 2 d after emergence. Therefore, the youngest insects used for experiments were 3 d old. A total of 258 individual pairs of moths of different age combinations (Table 4.1) were set up for the duration of their lifespan in test cylinders. In this experiment, insects of different ages were exposed to each other to determine whether and to what extent age affected mating success, fecundity and fertility.

**Table 4.1** Age combinations of pairs and sample size used to assess the effect of mating delay on reproductive fitness in *Cnephasia jactatana*

| Age (d) combinations of pairs | | No. of pairs |
|---|---|---|
| Female | Male | |
| 3 | 3 | 38 |
| 3 | 7 | 28 |
| 5 | 5 | 24 |
| 5 | 9 | 34 |
| 7 | 3 | 18 |
| 7 | 7 | 38 |
| 7 | 11 | 20 |
| 9 | 5 | 14 |
| 9 | 9 | 30 |
| 11 | 7 | 14 |

*Cnephasia jactatana* spermatophore consists of a corpus (almost spherical) and a collum as in most Tortricidae (Benz 1991). I dissected dead females under a dissecting microscope and measured the diameter of the corpus of the spermatophore using a micrometer eyepiece.

### 4.2.3 Statistical Analysis

A central composite design (CCD), also called response surface (Box and Draper 1987), was used to determine whether mating delay affected reproductive

performance. The CCD is a more efficient way to arrange samples than a full factorial design. It assumes a functional relationship between input variables (ages) and outputs (e.g. fecundity, fertility), and provides the possibility to infer effects of other input combinations or establish the input variable values for a fixed output (Box and Draper 1987). The response surface predicts those outputs by fitting functions rather than means. The estimated value of the response variable logit $\hat{g}(x)$ is given by the polynomial equation:

$$\hat{g}(x) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x^2_1 + \beta_{22} x^2_2 + \beta_{12} x_1 x_2 + \epsilon$$

where $\hat{g}(x)$ is the estimated value of log (fecundity) or log (fertility), $\beta_0$ is the intercept on $y$ of $\hat{g}(x)$, $x_1$ = male age (days), and $x_2$ = female age (days). The rate of change (slopes) associated with male age, female age and their interactions is represented by $\beta_1$, $\beta_2$, and $\beta_{12}$, slopes of the quadratic effect on male age and female age are presented by $\beta_{11}$ and $\beta_{22}$, and $\epsilon$ represents the error term.

Where appropriate, a Poisson or a normal distribution was used to test fecundity, fertility and spermatophore size. Only those couples that laid eggs or transferred a spermatophore were used in the analysis. Fertility rate and the probability of laying fertile eggs or mating success (transferring a spermatophore) were tested using a binomial distribution where the oviposition of fertile eggs or the presence of a spermatophore was considered as a success. Only significant terms, after running the full regression model, were kept in the final models. The coefficient of regression for each model was computed according to Hosmer and Lemeshow (2000). The body weight was used as a covariate in the analyses for fecundity, fertility, and spermatophore size. A log likelihood ratio test (McCullagh and Nelder 1989) was used to determine whether mating delay had differential effects between sexes. All ages are days from emergence.

### 4.2.4 Results

#### 4.2.4.1 Effect of Mating Delay on Reproductive Fitness and Spermatophore Size

The probability of successful mating, evidenced by the presence of a spermatophore in the female body, significantly decreased as the age of both sexes increased ($F = 8.02$; $df = 4, 251$; $P < 0.0001$; $R^2 = 0.1126$) (Figure 4.1 A). For example, > 70% of 3- or 5-d-old pairs successfully mated while only ≈50% of 7- or 9-d-old ones achieved mating. The model predicted that when either sex was 11 d old the probability of successful mating was reduced to ≈ 30%. According to the likelihood ratio test aging affected males more severely than females in terms of mating success ($\chi^2 = 19.56$, $df = 2$, $P < 0.005$) (Figure 4.1 B).

Mating delay also significantly reduced the number of pairs that were able to produce fertile eggs ($F = 17.33$; $df = 5, 252$; $P < 0.0001$; $R^2 = 0.2157$). For example, 3-d-old pairs had significantly higher probability of laying fertile eggs than 5-, 7- or 9-d-old pairs (Figure 4.2 A). Figure 4.2 B showed a different response by males and females to mating delay, but the difference was not significant ($\chi^2 = 4.61$; $df = 2$; $P = 0.10$).

Fecundity was influenced by mating delay with 9-d-old pairs producing significantly fewer eggs than 3-, 5- or 7-d-old pairs ($F = 17.64$; $df = 5, 215$; $P < 0.0001$, $R^2 = 0.2909$) (Figure 4.3A). The predicted male fecundity linearly decreased as the male mating age increased but the predicted female fecundity presented a quadratic pattern where after an increase (until 4 d old) it decreased sharply (Figure 4.3 B). It is indicated that female aging had significantly more effect on fecundity than male aging ($\chi^2 = 291$; $df = 2$; $P < 0.005$).

Fertility also significantly decreased as the age of either sex increased ($F = 32.94$; $df = 4, 216$; $P < 0.005$; $R^2 = 0.3788$) (Figure 4.4 A). However, the effect of female aging on fertility was predicted to be almost 3 times greater than male aging ($\chi^2 = 49.90$; $df=2$; $P < 0.005$) (Figure 4.4 B).

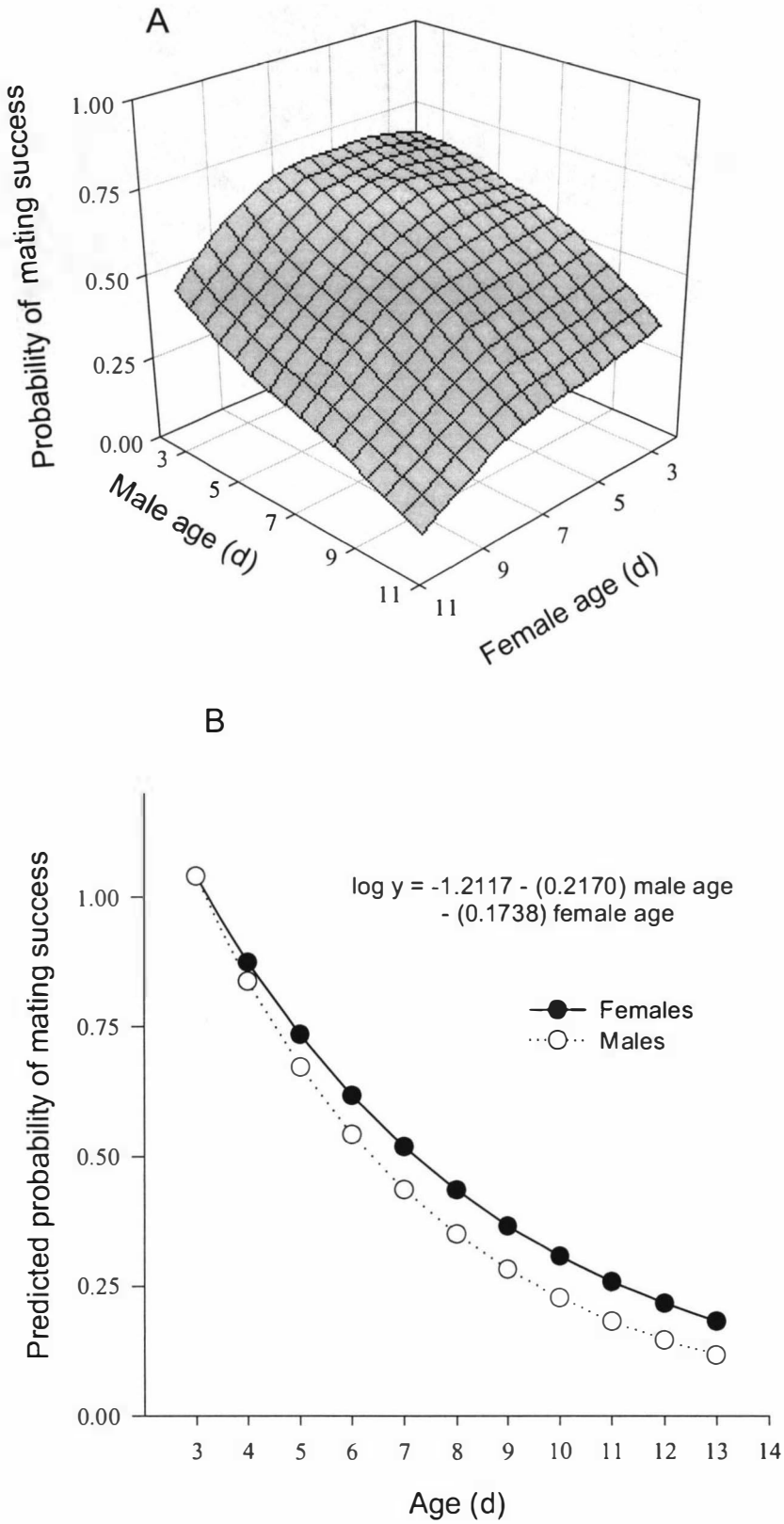


**Figure 4.1** Effect of mating delay on mating success of *Cnephasia jactatana*: A, probability of mating success in pairs of different age combinations (n = 258); and B, predicted mating success of males and females of different ages.

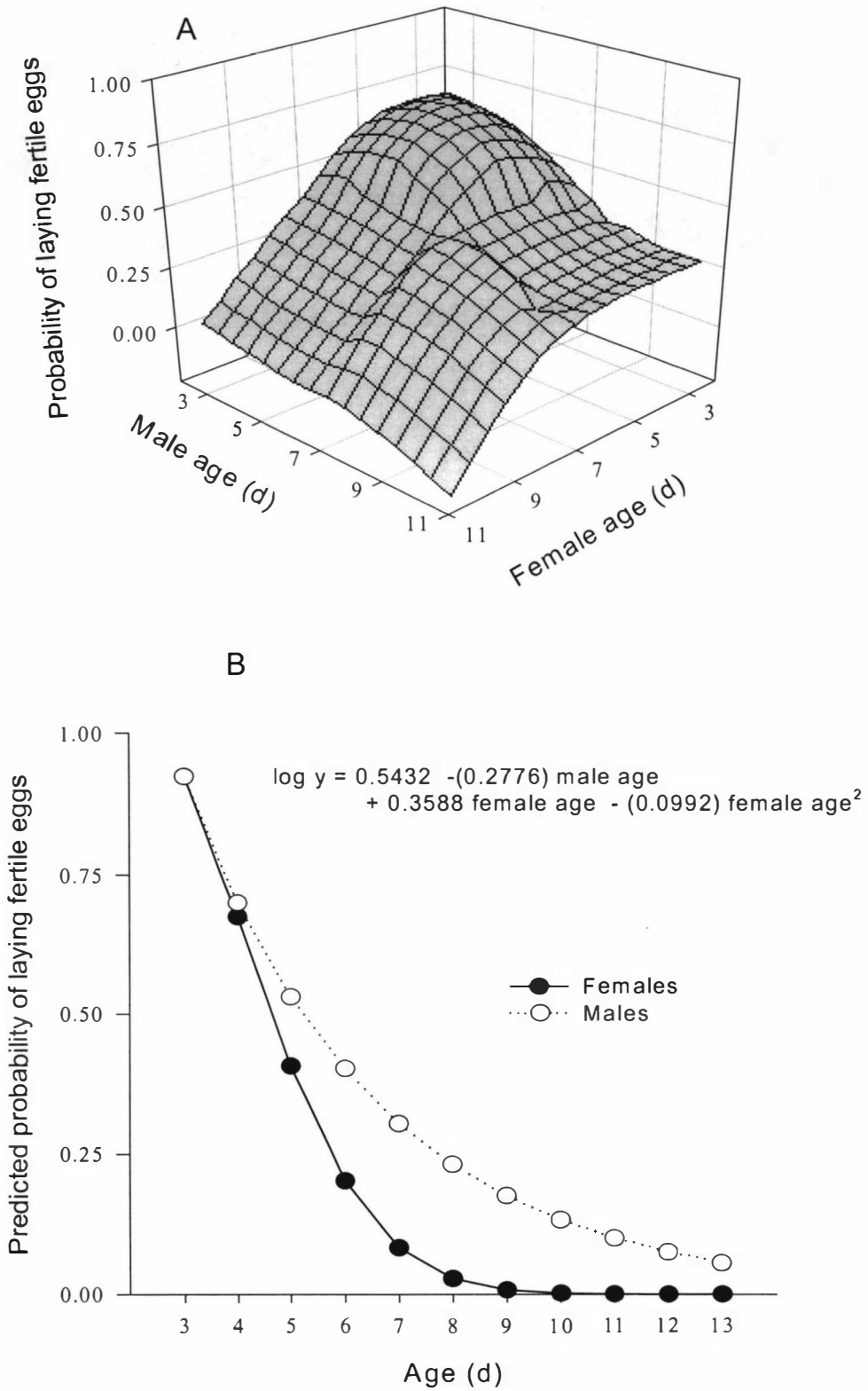


**Figure 4.2** Effect of mating delay on probability of production of fertile eggs in *Cnephasia jactatana*: A, probability of production of fertile eggs by pairs of different age combinations (n = 258); and B, predicted sex and age effect on probability of production of fertile eggs.

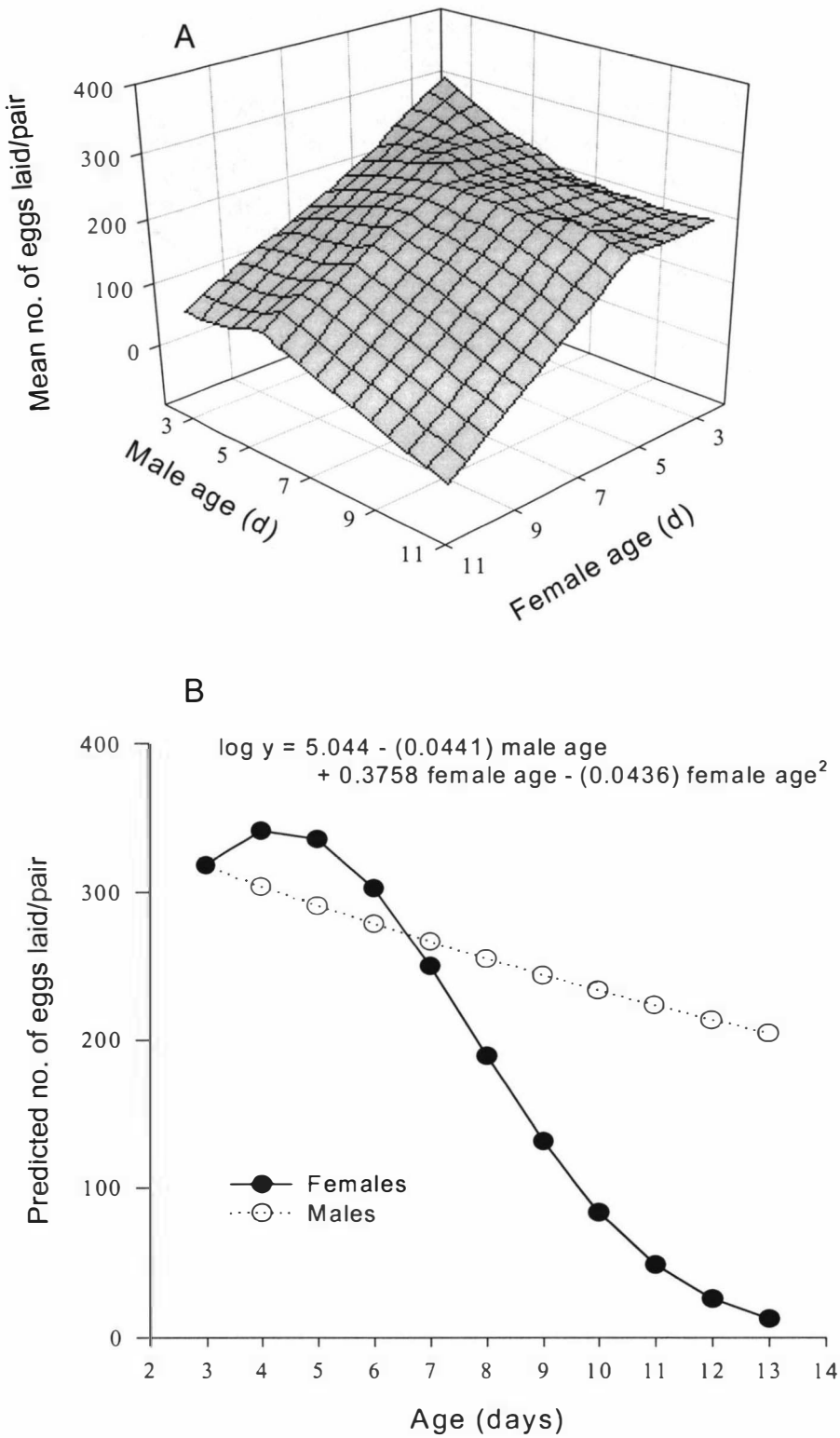


**Figure 4.3** Effect of mating delay on fecundity of *Cnephasia jactatana*: A, mean number of eggs laid by pairs of different age combinations (n = 221); and B, predicted sex and age effect on the number of eggs laid.

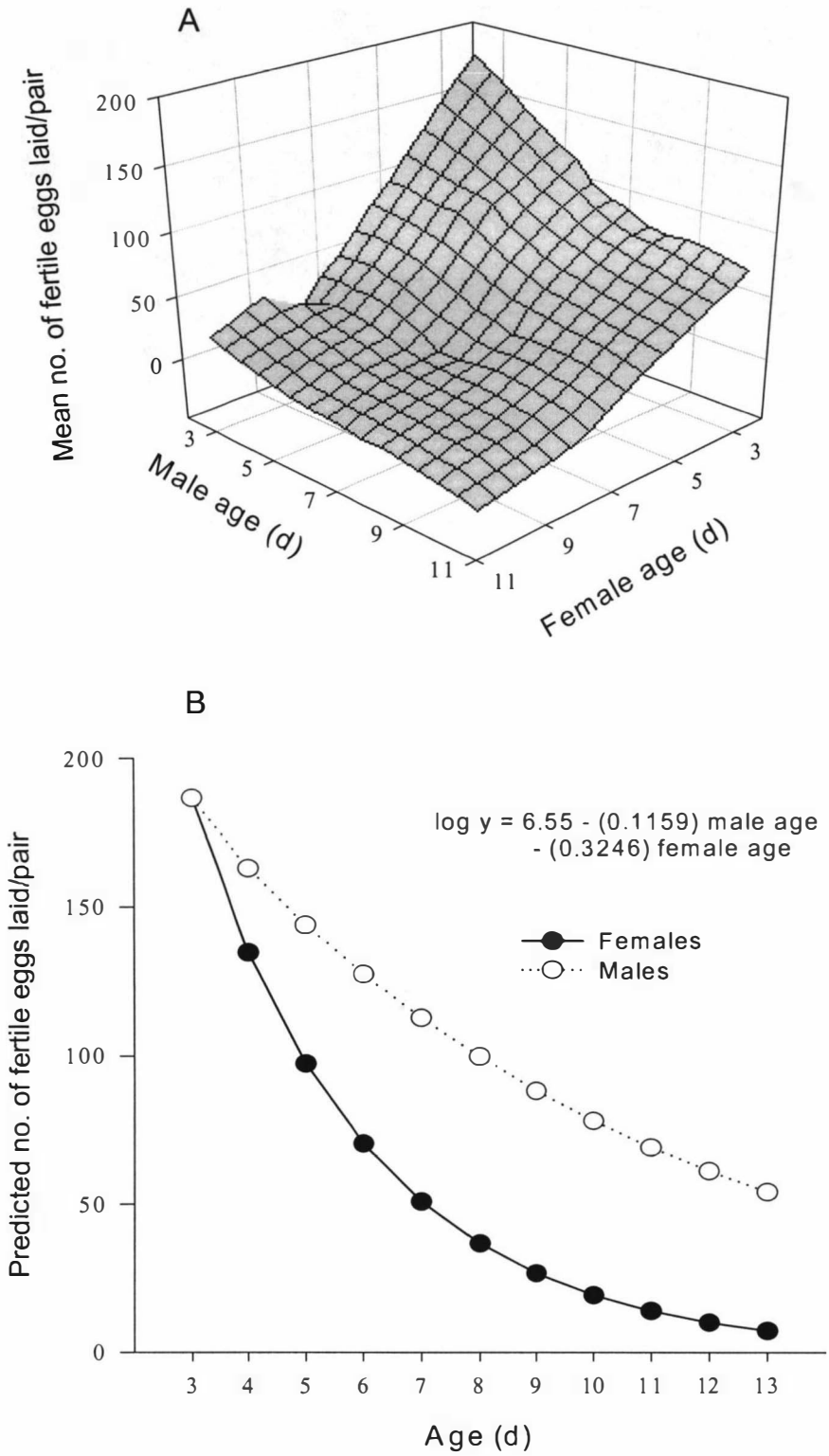


**Figure 4.4** Effect of mating delay on fertility of *Cnephasia jactatana*: A, mean number of fertile eggs laid by pairs of different age combinations (n = 221); and B, predicted sex and age effect on the number of fertile eggs laid.

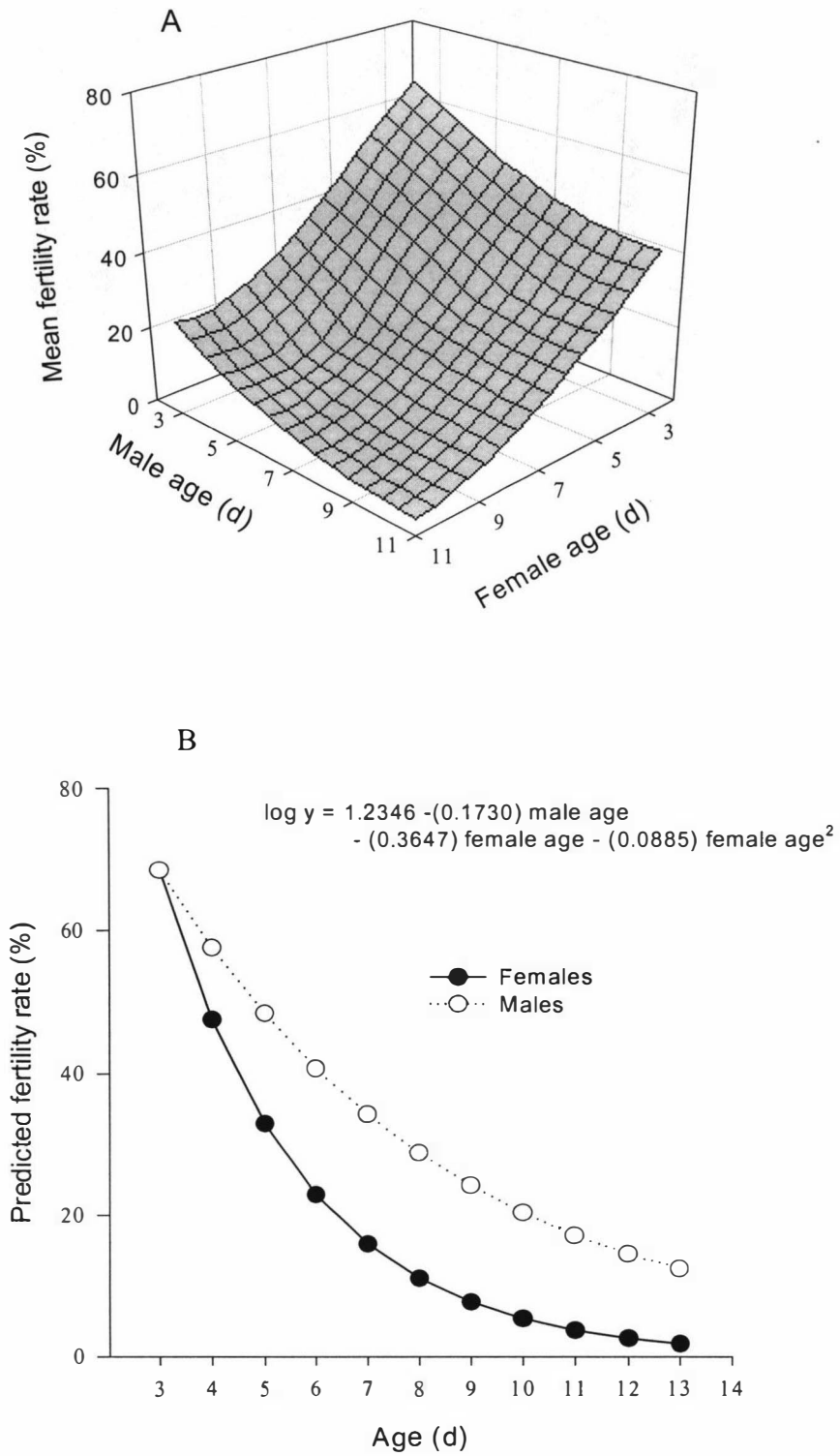


**Figure 4.5** Effect of mating delay on fertility rate of *Cnephasia jactatana*: A, mean fertility rate in pairs of different age combinations (n=221); and B, predicted sex and age effect on fertility rate.

Mating delay of both sexes also significantly affected fertility rate ($F = 25.77$; $df = 4, 216$; $P < 0.0001$; $R^2 = 0.3231$) (Figure 4.5 A). For example, the pairs that mated at 6 d old had their fertility rate ≈80% lower than those that mated at 3 d old. Fertility rate decreased almost linearly as male age increased and exponentially as female age increased, indicating that reduction in fertility rate associated with aging in females was significantly greater than in males ($\chi^2 = 7.73$; $df = 2$; $P < 0.01$) (Figure 4.5 B).

No relationship was found between the spermatophore size and the age of both sexes at mating ($F = 1.30$; $df = 4,145$; $P > 0.05$). The average spermatophore size was 0.99 ± 0.007 mm.

The number of pairs achieving a second mating decreased as the age of the insects increased. For example, ≈26, 18 and 17% of pairs where both sexes were 3, 5 or 9 d old achieved a second mating.

#### 4.2.4.2 Effect of Mating Delay on Oviposition Pattern

Oviposition was adversely affected by mating delay. Pairs where both insects were 3 d old at time of mating had a curvilinear oviposition pattern, producing a total of 319 ± 5.91 eggs. However, pairs formed by a 3-d-old female × a 7-d-old male and by a 7-d-old female × a 3-d-old male produced significantly fewer eggs, being only 184 ± 15.7 and 158 ± 17.2, respectively ($F = 17.64$; $df = 5, 215$; $P < 0.05$) (Figure 4.6).

### 4.2.5 Discussion

Previous studies show that mating delays substantially reduce the reproductive potential in some tortricid moths such as *E. postvittana* (Foster and Ayers 1996), *Argyrotaenia citrana* (Knight 1996), *Cy. pomonella* (Vickers 1997), and *Pandemis* spp. (Knight and Turner 1999). However, these studies evaluated the effect of mating delay by pairing one sex of various ages with another of fixed age, or by pairing insects with the same ages. This approach cannot precisely quantify which sex is more severely affected by mating delays and what age interactions

occur between sexes, information of which is important for the development of pest management strategies and study of insect physiology.

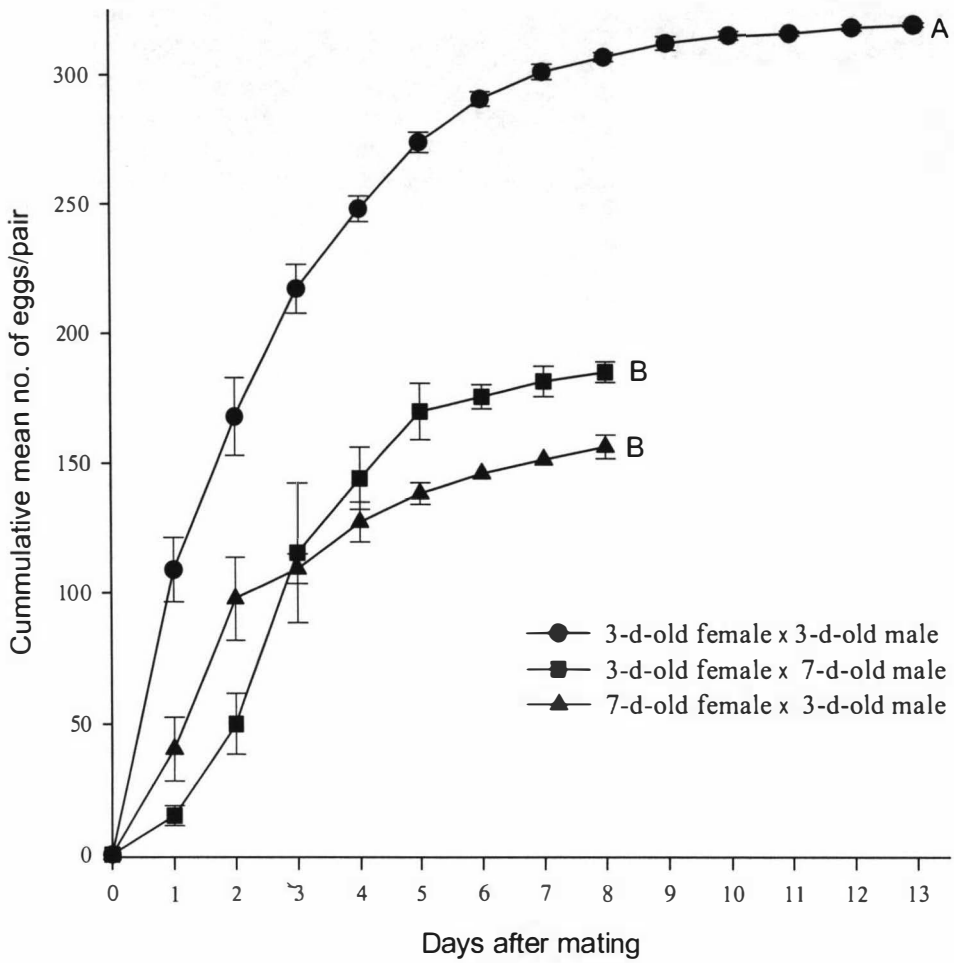


**Figure 4.6** Oviposition patterns of three age combinations: 3-d-old females × 3-d-old males (n = 31), 3-d-old female × 7-d-old males (n = 22), and 7-d-old females × 3-d-old males (n = 16). Lines with the same letter are not significantly different ($P > 0.05$). Bars are SE.

In the present study of *C. jactatana*, I used a different methodology by setting up pairs with a series of age combinations. This approach has allowed me to determine whether and to what extent the mating delay affected the reproductive performance as well as which sex was more severely affected. It is suggested that the reproductive fitness is significantly affected by mating age of both sexes and that females are more severely affected by mating delay than males in terms of

reproductive fitness in *C. jactatana* (Figure 4.3 B and Figure 4.4 B), which coincides with Foley's (1985) conclusion that the senescence process influences females more than males in *Phthorimaea operculella*. With increasing age in virgin females, fewer nutrients may be placed within the maturing ova and even reabsorbed from the ova (Proshold et al. 1982). Senescence also may diminish females' ability to transport or store sperm (Proshold 1996). Furthermore, like many other moths such as *Cy. pomonella* (Karalius and Buda 1995, Vickers 1997) and *E. postvittana* (Foster and Ayers 1996), virgin *C. jactatana* females lay increasing number of unfertilised eggs as the age increases, reducing the number of eggs that can potentially be fertilised.

The *C. jactatana* sexual maturation period is ≈2 d after emergence (Section 3.4). Our observations show that best reproductive performance can be achieved if females mate when 3 and 4 d old. If mating disruption in an orchard delays the female's mating until she is 5 d old and we assume that she mates with an insect of the same age, their fecundity would decrease from 186.96 ± 9.99 eggs to 132.92 ± 16.85 eggs, a reduction of 27%, and until she is 7 d old, to 20.67 ± 3.61 eggs, an 88% reduction. It is thus suggested that it is necessary to delay females' mating for at least 2 to 3 d after they are sexually mature to achieve control. In addition, mating disruption potentially increases moth activities associated with mate location and thus chances to be attacked by natural enemies (Jones and Aihara-Sasaki 2001).

This section has demonstrated that delaying mating for 2-3 days severely reduces the reproductive potential of *C. jactatana*. It is suggested that mating disruption may have potential as a control technique of this pest.

## 4.3 Effect of Female Remating on Reproductive Fitness of *Cnephasia jactatana*

### 4.3.1 Introduction

Understanding the factors controlling polyandry is important not only in terms of behaviour and ecology of the species, but also from an applied perspective. For example, grasping the motives for multiple mating and fathoming its frequencies may help us determine how far polyandrous females could compete with the female sex pheromone traps employed for mating disruption or population monitoring (Sadek 2001).

Both sexes of *C. jactatana* can mate more than once, with males being able to mate more times than females. In this section, I tested (1) whether and to what extent the multiple mating affected the fecundity and fertility of *C. jactatana* females, (2) how such effect varied according to larval nutritional conditions and male virginity, and (3) the factors that affected the female remating behaviour.

### 4.3.2 Materials and Methods

#### 4.3.2.1 Insects

Both individually-reared and mass-reared insects (Section 3.3.2.1) were used for these experiments. Both rearing systems used the same artificial diet developed by Singh (1983) and under the same environmental conditions. Pupae were separated by sex and kept individually in glass vials until adult emergence to ensure virginity. Female and male pupae were kept in separate rooms until the experimental day. All experiments were carried out under reverse-light conditions (Section 3.2.3).

#### 4.3.2.2 Influence of Remating and Larval Nutrition on Female Reproductive Fitness

To determine whether remating with the same male enhanced the female's fecundity and fertility and how this effect varied according to larval rearing conditions (nutritional levels), I set up 86 pairs from mass-rearing system and 176

pairs from individually-reared system. All pairs consisted of 3-d-old moths with each pair being individually caged in test cylinders. Egg masses were collected daily and fertility assessed as described in Section 3.2.2 Oviposition period was recorded.

#### 4.3.2.3 Influence of Male Virginity on Female Reproductive Fitness

Only individually-reared insects were used in this experiment. To determine whether male virginity had any effect on female reproductive performance, I undertook two treatments: (1) a female and a male were caged for life and the female had the opportunity to remate with the same male (n = 176) and (2) a female had the opportunity to remate with different virgin males (n = 31). For the second treatment, a 3-d-old male was provided for a female (started from 3 d old) for the duration of the dark period (8 h) each day until the female was 12 d old. Egg masses were collected daily and fertility assessed as described in Section 3.2.

#### 4.3.2.4 Influence of Bodyweight, Spermatophore Size and Mating Length on Female Remating Behaviour

To determine whether the bodyweight, the size of the first spermatophore and length of the first mating affected female remating behaviour, I weighed all pupae before experiments, dissected dead females and measured the diameter of the spermatophore corpus using a dissecting microscope, and recorded the first mating for 31 pairs using and electronic clock (DSE, New Zealand). According to Drummond (1984) and Howell (1991), the number of spermatophores found in the bursa copulatrix of a female is an indirect indication of the number of matings a female has achieved. This method was employed to estimate the number of matings each female achieved.

### 4.3.3 Statistical Analysis

An analysis of covariance (ANCOVA) followed by a least squared means test was used to compare reproductive parameters of mass- and individually-reared pairs. The interaction between diet and number of matings was tested on a 2-way ANCOVA. Pupal weight was used as a concomitant variable. The remating rate of females from different rearing systems was compared with a z test (Freedman et al. 1998). A regression analysis was used to test for fertility rate decline over

oviposition period. The effect of body weight and the first spermatophore size on female remating behaviour was tested using an analysis of variance (ANOVA) followed by a least significant difference test. The remaining data were analysed with a *t* test. Fertility rate was arcsine transformed prior to analysis (Steel et al. 1997).

### 4.3.4 Results

#### 4.3.4.1 Influence of Remating and Larval Nutrition on Female Reproductive Fitness

Remating with the same males significantly increased fecundity and fertility of females in both rearing systems (Table 4.2). However, mass-reared females benefited significantly more from remating than individually-reared females in both fecundity ($F$ = 3.49; df = 1,255; P = 0.04) and fertility ($F$ = 3.89; df = 1,255; P = 0.04). For example, remating increased fecundity and fertility by 140 and 141 eggs, respectively, for insects from the mass-rearing system, while for those from the individual-rearing system, the increase in fecundity and fertility due to remating was only 66 and 58 eggs, respectively (Table 4.2). For both rearing systems fertility rate did not differ between once-mated and remated pairs. In mass-reared insects the number of once-mated pairs was significantly greater than that of remated pairs (z = 10.28; P < 0.0001) while in individually-reared insects, the opposite was the case (z = 6.39; P < 0.0001). Remating significantly increased daily fertility for mass-reared females but did not for individually-reared females (Table 4.2).

Decline in daily fertility rate of mass-reared insects followed a quadratic pattern while a linear equation described fertility rate changes for individually-reared pairs (Figure 4.7). Fertility rate of mass-reared insects was > 50% for the first 7 d after the first mating and thereafter quickly dropped to < 20%, 11 d after the first mating. On the contrary, individually-reared insects gave > 80% fertility rate during the same period and the lowest fertility rate (67.88%) occurred 18 d after the first mating. In addition, individually-reared females had significantly longer oviposition period (13.9 ± 0.39 d) than mass-reared ones (6.96 ± 0.29 d) ($t$ = 11.5; df = 260; P < 0.0001).

**Table 4.2** Reproductive parameters (mean ± SE) of mass- and individually-reared *Cnephasia jactatana* permanent pairs

| Variable | Mass-reared insects | | Individually-reared insects | | F | P |
|---|---|---|---|---|---|---|
| | Mated once (n = 77) | Remated (n = 9) | Mated once (n = 58) | Remated (n = 118) | | |
| Fecundity | 279 ± 20c | 419 ± 58b | 533 ± 23b | 599 ± 16a | 53.2 | <0.01 |
| Fertility | 194 ± 22c | 335 ± 66b | 450 ± 26ab | 508 ± 18a | 39.6 | <0.01 |
| Fertility rate (%) | 59.4 ± 4.0b | 74.2 ± 10ab | 83.0 ± 3.1a | 81.9 ± 2.2a | 12.6 | <0.01 |
| Daily fertility | 47.5 ± 2.0b | 60.1 ± 5.9a | 43.6 ± 2.3b | 44.1 ± 1.6b | 2.7 | <0.05 |
| Size of the first spermatophore (mm) | 0.84 ± 0.03a | 0.96 ± 0.06a | 0.95 ± 0.01a | 0.94 ± 0.01a | 2.4 | >0.05 |

Means in rows followed by the same letter are not significantly different (ANCOVA, P> 0.05)

#### 4.3.4.2 Influence of Male Virginity on Female Reproductive Fitness

The analysis showed that females that remated with either the same males or virgin males gave similar fecundity (614 ± 16 for experienced males and 640 ± 36 for virgin males; $t$ = 0.63; df = 136; P > 0.05), fertility (520 ± 20 for experienced males and 589 ± 42 for virgin males; $t$ = 1.36; df = 136; P > 0.05) and fertility rate (81.8 ± 2.2 for experienced males and 90.6 ± 2.8 for virgin males; $t$ = 1.64; df = 136; P > 0.05). However, females that remated with virgin males had significantly higher daily fecundity (54.8 ± 3 egg/d) ($t$ = 2.91; df = 136; P = 0.004) and shorter oviposition period (12 ± 0.5 d) ($t$ = 2.67; df = 136; P = 0.0085) than those that remated with the same males (44.8 ±1 egg/d and 14.59 ± 0.4 d, respectively).

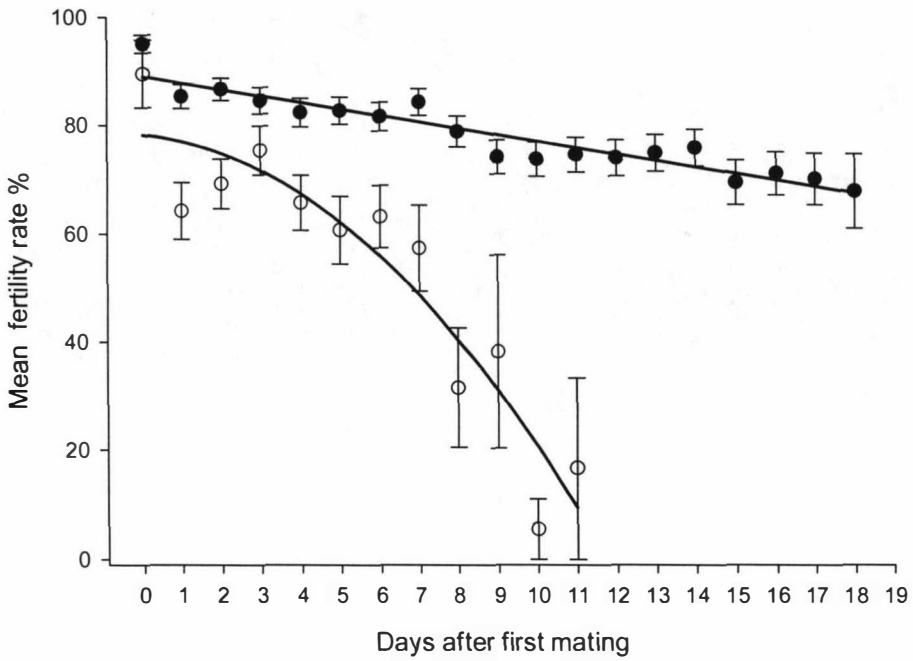


**Figure 4.7** Daily fertility rate of mass-reared (empty dots) and individually-reared (solid dots) *Cnephasia jactatana* females that had continuous access to the same males. Bars are SE.

The fertility rate of females that had continuous access to virgin males did not significantly decrease as time (d) increased no matter whether remating occurred ($F = 3.39$; $df = 1,11$, $P = 0.0928$ for once and $F = 1.30$; $df = 1,13$, $P = 0.2752$ for twice mated females, respectively).

#### 4.3.4.3 Influence of Bodyweight, Mating Length and Spermatophore Size on Remating Behaviour

ANOVA revealed that females that remated were significantly heavier than females that did not remate regardless of male virginity ($F = 5.36$; $df = 2, 202$; $P = 0.0014$) (Figure 4.8). However, male weight did not influence whether or not a female remated ($F = 0.87$; $df = 2,202$; $P > 0.05$).

The length of the first mating did not influence the remating behaviour of females because the first mating of females that remated ($n = 21$, $70.4 \pm 4.05$ min)

was similar to that of females that did not remate (n = 10, 72.5 ± 3.27 min) ($t$ = 0.33; df = 29; P > 0.05).

The size of the first spermatophore did not affect whether or not a female remated as the first spermatophores in females that mated twice (0.96 ± 0.02 mm) were of similar size to those that mated only once (0.99 ± 0.15 mm) (F = 1.16; df =3, 202; P > 0.05). Furthermore, insects from both rearing systems produced a first spermatophore of similar size (Table 4.2).

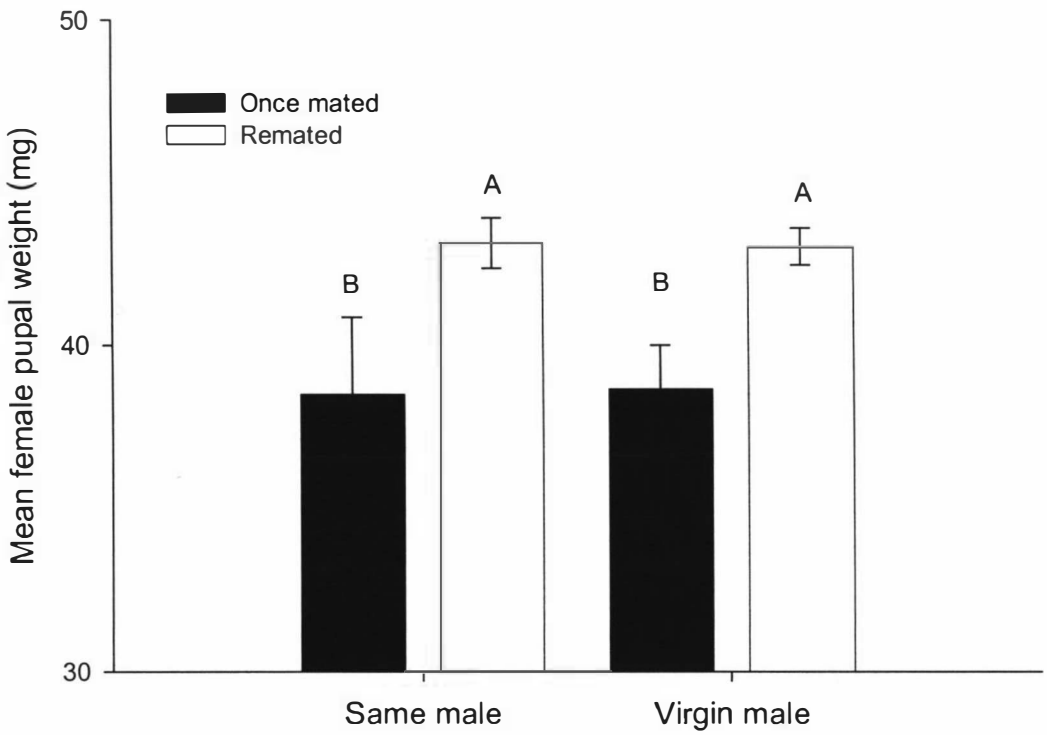


**Figure 4.8** Effect of *Cnephasia jactatana* female bodyweight on her remating behaviour. Error bars are SE. Bars with the same letter are not significantly different (ANOVA, P > 0.05)

### 4.3.5 Discussion

This study shows that remating increases female reproductive capacity of *C. jactatana* in both rearing systems, and that mass-reared insects benefit more from remating than individually-reared ones in terms of fecundity and fertility. The increase of fecundity and fertility in remated females may be attributed to the transference during mating of more sperm and male-derivate substances (MDS),

which contribute to higher fertility and energetic reserves for reproduction and body maintenance (Wiklund et al. 1993, Bissoondath and Wiklund 1996a, Baker et al. 2001).

Boggs' model (1990) for the role of male-donated nutrients in insect reproduction predicts that remating should be more important in species where females emerge with few or no mature eggs, adults feed on a low protein diets and larvae are under food stress, such as *C. jactatana*. However, despite significantly higher fecundity and fertility produced by remating in mass-reared moths, they remated significantly less frequently than individually-reared moths. Bergström et al. (2002) and Bergström and Wiklund (2002) found similar results for pierid *Pieris napi*. Since the production of an ejaculate is physiologically costly and compromises great energetic reserves (Bissondath and Wiklund 1996a), males developed under food stress may have more difficulties to accumulate enough reserves to produce a second ejaculate. This may explain the lower remating frequency and the rapid decline on fertility rate over time in the mass-reared pairs. Gage and Cook (1994) demonstrated that pyralid *Plodia interpunctella* males developed under food competition suffered resource restrictions for spermatogenesis and transferred reduced sperm numbers to females at mating.

The fact that remating significantly increased daily fertility for mass-reared *C. jactatana* could be interpreted as mass-reared males, due to their limited reserves, not being able to produce or transfer enough sperm in the first mating. However, more sperm and MDS are produced over time after the first mating (Giebultowicz and Brooks, 1998). If remating occurs, the male could replenish the female with more sperm, boosting her daily fertility.

Oberhauser (1997) found that larger *Danaus plexippus* females had a longer oviposition period as a result of more energy reserves obtained during the larval stage. Individually-reared *C. jactatana* are heavier than mass-reared ones (Section 3.3) and are expected to live longer. Heavy females usually have more eggs (Section 3.6). The benefit of this is to keep their daily oviposition rate constant during a longer period of time, increasing the offspring survival.

The fertility rate of females that had continuous access to virgin males did not decrease as time (d) increased no matter whether remating occurred. This suggests that females have control over whether a remating is necessary to maximise her fitness.

Unlike some other species such as *Callosobruchus maculatus* (Savalli and Fox 1999) and *Cordylochernes scorpioides* (Newcomer et al. 1999), *C. jactatana* females that remate with either virgin or mated males had similar reproductive potential. Similar results were obtained for *Cyrtodiopsis dalman* (Baker et al. 2001) and *Spodoptera litoralis* (Sadek 2001). It is suggested that to significantly suppress the *C. jactatana* population, we should reduce the number of males mating with females to the minimum because females remating with either experienced or virgin males would have similar reproductive output.

However, *C. jactatana* females that had a second mating with virgin males gave higher daily fecundity and completed egg laying in a shorter period of time, indicating that remating with virgin males accelerates egg production and maturation.

The present study shows that the number of matings was positively correlated with *C. jactatana* female weight (Figure 4.8). van Dongen et al. (1999) and Wedell and Cook (1999a) also found that heavier females achieved more matings. This could be interpreted as that heavy females with inherently greater egg-laying capacity are able to produce more eggs and so they need to obtain more sperm and MDS from remating to achieve their maximal reproductive gains (Shapiro et al. 1994).

It is reported that long matings may result in the transference of larger amounts of sperm and MDS and consequently larger spermatophores (Rutowski et al. 1987, Wang and Millar 1997). Therefore, short matings have been considered a reason for remating (Bissoondath and Wiklund 1996a, Sakurai 1998). However, my results showed that the length of the first mating in females that remated was similar to those that did not remate, ruling out short mating as a reason for remating.

Mass-reared males are lighter than individually-reared ones (Section 3.3) but both produce spermatophores of similar size. Therefore, mass-reared males compromise more resources in relation to their body weight for spermatophore production than individually-reared males. This phenomenon indicates a

reproductive disadvantage for small males (Bissoondath and Wiklund 1996a). Finally, my study shows that larval rearing conditions did not influence the size of the first spermatophore nor did the size of the first spermatophore influence female remating behaviour. This suggests that the spermatophore size does not play an important role in the remating behaviour of *C. jactatana* females.

## 4.4 Effect of Male Remating on Reproductive Fitness of *Cnephasia jactatana*

### 4.4.1 Introduction

Section 4.3 reported how diet and female remating affected female reproductive fitness. However, there has been no report on how male mating history influences the reproductive success of *C. jactatana*, information of which is useful for understanding the mating system and development of integrated pest management tactics for this species.

This section reports the remating behaviour of *C. jactatana* males and the effect of their mating history on the female reproductive fitness.

### 4.4.2 Materials and Methods

Insects were obtained from the individual rearing system (Section 3.3.2.1). Pupae were separated by sex and weighted as described in Section 3.2. Female and male pupae were kept in separate rooms until the experimental day. All experiments were carried out in a reverse-light regime (section 3.2.3).

To determine how many times a male could mate in his lifespan and whether the male fecundity changed with the number of matings achieved, I introduced a 3-d-old virgin male and a 3-d-old virgin female into a test cylinder. After mating, the male was removed and kept in a glass vial. The male was then offered a 3-d-old virgin female in the test cylinder every 24 h until he failed to mate for 2 consecutive days. I used a total of 70 males and followed up the fecundity and fertility of 180 mated females. Males that did not achieve any mating were discarded. Eggs were collected daily and fertility assessed as in Section 3.2. Dead females were dissected and the diameter of the spermatophore corpus measured under a dissecting microscope. The following parameters for each mating were recorded: activation time (between the introduction of the male and the start of courtship display by the male), courtship length, mating length, and spermatophore size. I also recorded each mated female's fecundity, fertility and oviposition period.

To test whether a male could mate more than once on the same day, I caged a 3-d-old virgin male with three 3-d-old virgin females for the duration of the dark period (8 h) in a test cylinder and recorded the number of females the male mated with. To differentiate potential partners, I marked the dorsal thorax of 2 of the potential mates with a correction pen (Mitsubishi pencil Co., Ltd. Japan). The potential partners to mark were selected randomly. At the end of the 8 h period, females were dissected and spermatophores measured as above. I observed 69 quartets in total.

Data on the female reproductive fitness and spermatophore size were analysed using orthogonal contrasts preceded by analysis of covariance (ANCOVA) (Steel et al. 1997). Activation, courtship and copula time were regressed over number of matings. A paired *t* test was used to compare the size of the first and the second spermatophore delivered within 8 h. A *t* test was used to compare the size of the second spermatophores delivered 24 h after the first mating and the second spermatophore delivered within 8 h. The marking procedure did not affect the probability of being mated (Binomial test, $P > 0.05$).

### 4.4.3 Results

#### 4.4.3.1 Male Remating Behaviour

The results show that with the increase of the number of matings, the percentage of *C. jactatana* males that achieved further matings decreased (Figure 4.9). If males had a 24-h recovery period between matings, > 50% of them were able to mate 4 times and ≈ 25% could achieve 6 matings in their lifespan (Figure 4.9). However, only 4 out of 69 males achieved a second mating within an 8-h period.

Males that managed to mate twice within 8 h produced a second spermatophore (0.51 ± 0.05 mm) 45% smaller than the first one (1.04 ± 0.01 mm) ($t = 7.14$, df = 3, P = 0.0028). However, the second spermatophore (0.72 ± 0.02 mm) transferred after a 24-h recovery period was 40% larger than that delivered within an 8 h period ($t = 3.37$, df = 37, P = 0.0018).

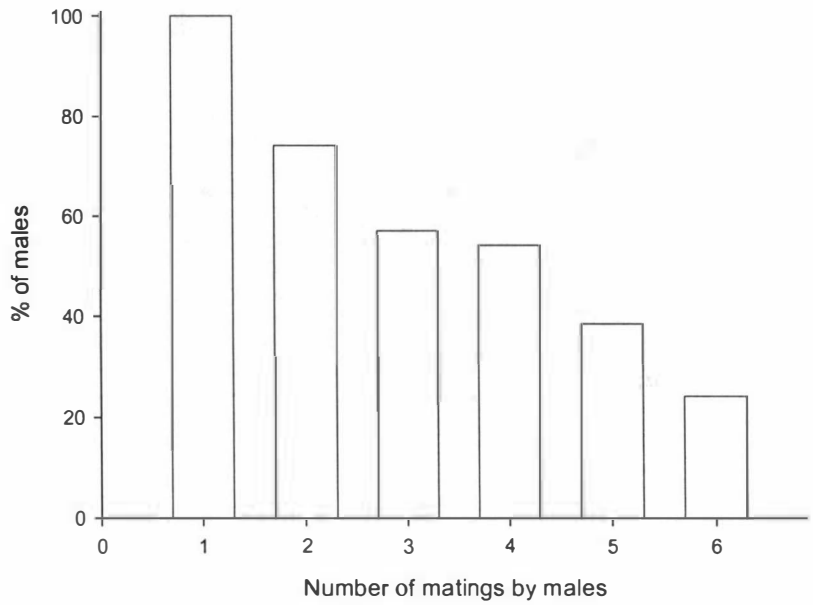


**Figure 4.9** Percentage of *Cnephasia jactatana* males achieving 1 to 6 matings

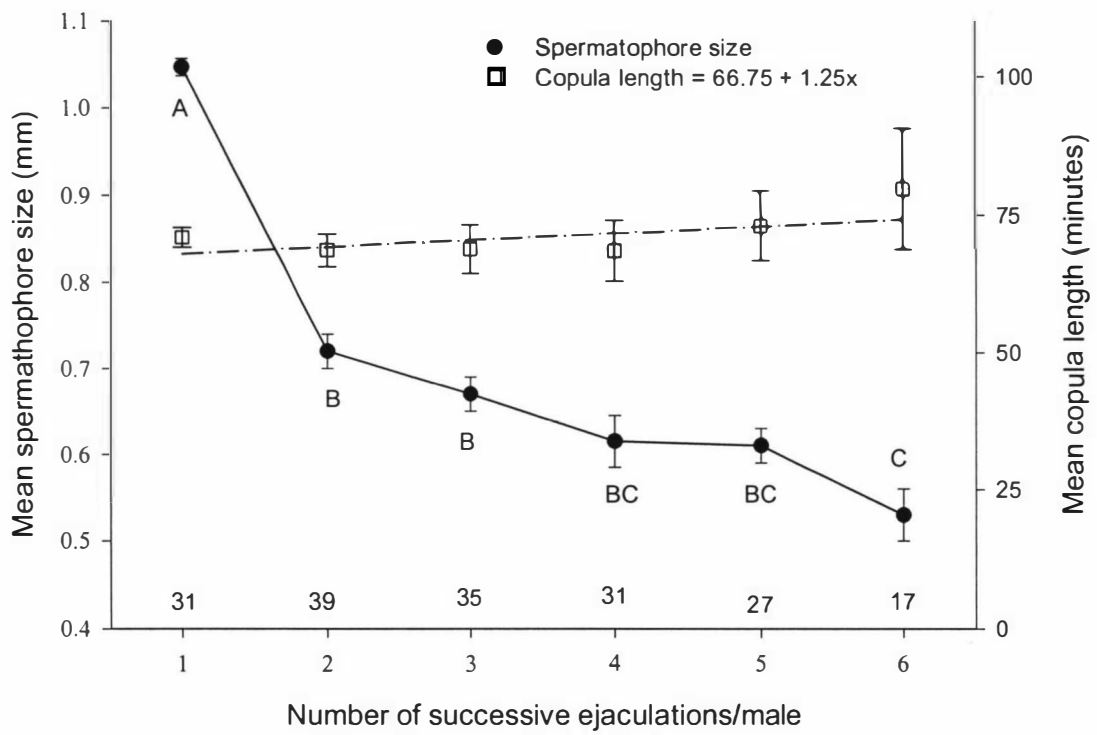


**Figure 4.10** Mean spermatophore size and mean copula length involved in *Cnephasia jactatana* males of different mating history. Mean spermatophore sizes followed by the same letter are not significantly different (ANCOVA, $P > 0.05$). Copula length regression is not significant ($F = 0.9$, $df = 1,176$, $P > 0.05$, $R^2 = 0.01$). Error bars are SE. Sample sizes are shown on top of x axis.

The size of spermatophores significantly decreased as the number of matings increased (ANCOVA; F = 13.05; df = 7, 108; P = 0.0001), with the sharpest decrease occurring between first and second matings (Figure 4.10). However, regardless of mating history males had similar copulation length (71.52 ± 1.92 min) (Figure 4.10).

Regression analysis showed that the length of courtship display (96.17 ± 9.17 min) remained similar for all matings but the activation time significantly increased as the number of matings increased (Figure 4.11).

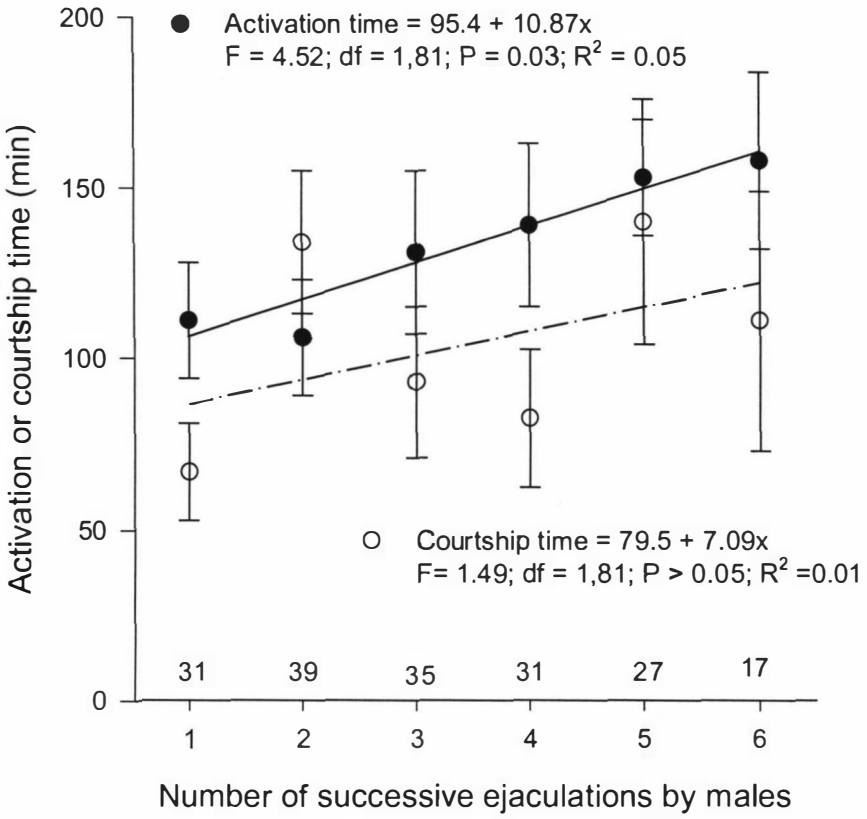


**Figure 4.11** Relationship between activation time or courtship time and the number of matings in *Cnephasia jactatana* males. Bars are SE. Sample sizes are shown on top of x axis.

#### 4.4.3.2 Effect of Male Mating History on Female Reproductive Fitness

Male mating history significantly affected female fecundity (ANCOVA; $F = 6.12$; $df = 5,111$; $P < 0.0001$) and fertility (ANCOVA; $F = 6.96$; $df = 5,111$; $P < 0.0001$) (Figure 4.12). Females mating with once- and twice-mated males suffered ≈ 20% and 27% reduction in fecundity, respectively, compared to females mating with virgin males. When females mated with males that had previously mated 3-5 times, their fecundity decreased by 40-50%. Relative to virgin males, females mating with once- and twice-mated males produced 22% and 30% fewer fertile eggs, respectively. About 50% reduction in fertility was observed for females that mated with males that had previously mated 3-5 times.

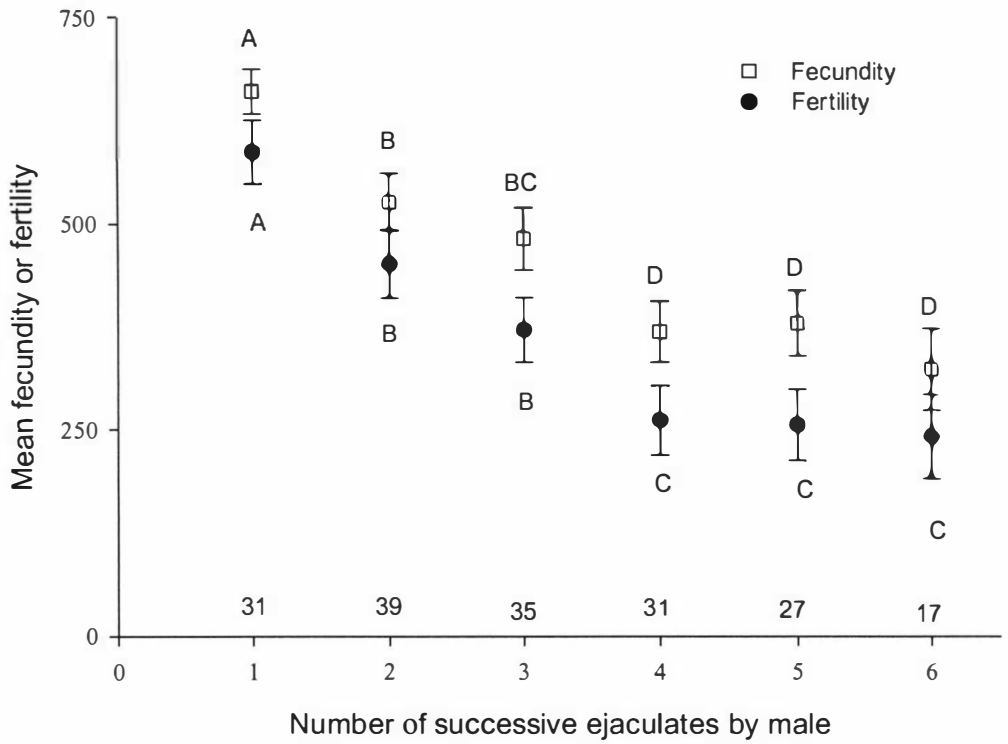


**Figure 4.12** Reduction on fecundity and fertility of *Cnephasia jactatana* females mating with males of different mating history. Fecundity and fertility means followed by the same letter are not significantly different (ANCOVA, $P > 0.05$). Error bars are SE. Sample sizes are shown on top of the x axis.

Male mating history also significantly affected oviposition period ($F = 3.42$; $df = 5,110$; $P = 0.0024$) (Figure 4.13). The oviposition period was on average 14 d if the female mated with a virgin male. This decreased to an average of 9-10 d if she mated with a 2- to 5-times mated male.

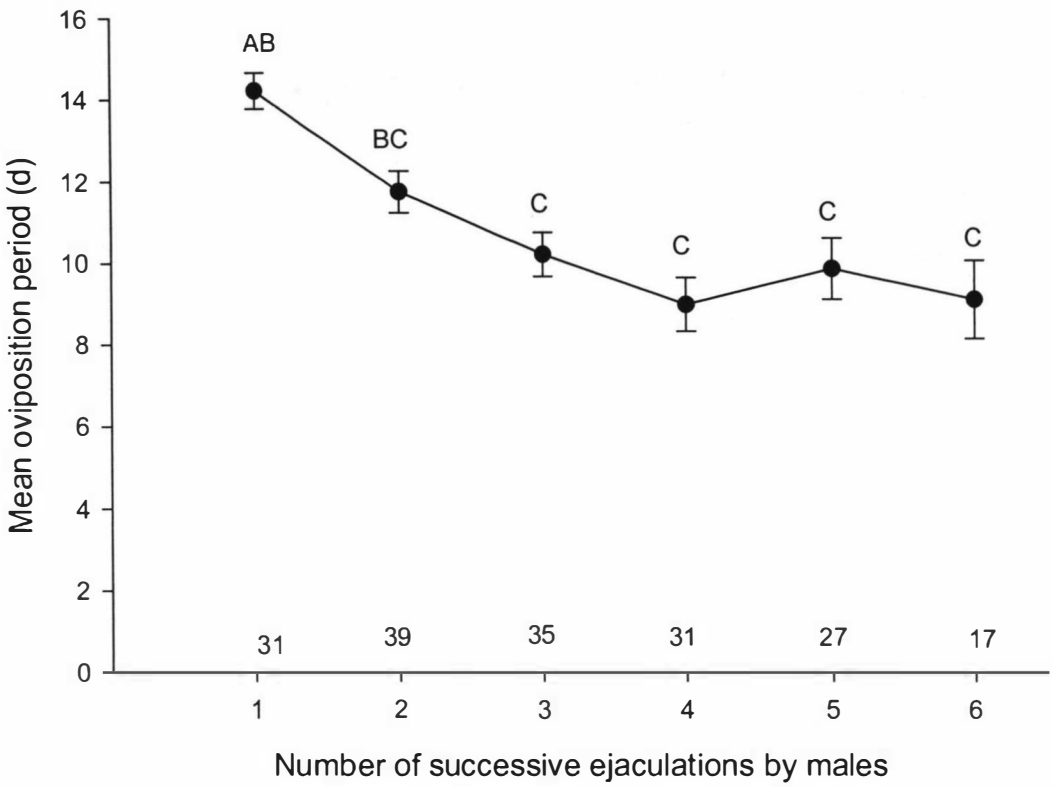


**Figure 4.13** Mean oviposition period of females mating with males of different mating history. Means followed by the same letter are not significantly different (ANCOVA, $P > 0.05$). Error bars are SE. Sample sizes are shown on top of x axis.

### 4.4.4 Discussion

Multiple matings are commonplace in lepidopteran males but male remating capacity varies between species. For example, *C. jactatana* males can mate up to 6 times; the tortricid *Zeiraphera canadensis* and *Cydia pomonella* males can achieve 5 and 18 matings, respectively (Howell et al. 1978, Carroll 1994), and the monarch butterfly *Danaus flexippus* males can mate 11 times (Oberhauser 1988).

In *C. jactatana* mating occurs exclusively during the dark period of a 24-h cycle (Section 3.4). The current results show that very few males are able to mate

twice within a 24-h cycle. It is suggested that most males require a recovery period for at least 24 h between successive matings. Like *C. jactatana*, the second spermatophore produced by the tortricid *Lobesia botrana* male within 24 h after the first mating is smaller than that delivered by the male that has had a 24-h recovery period after the first mating (Torres-Vila et al. 1995). These facts support the hypothesis that the production of ejaculates is costly and limited (Drummond 1984, Svärd 1985, Fitzpatrick and McNeil 1989, Svensson et al. 1998) and follows a circadian rhythm (Giebultowicz and Brooks 1998).

Unlike many other moth species [such as *Cy. pomonella* (Howell et al. 1978), *P. napi* (Kaitala and Wiklund 1995) and *P. rapae* (Bissonndath and Wiklund 1996a)] whose mating period increases with the increase of matings, *C. jactatana* males have similar mating periods for all matings. This behaviour is strategically important for *C. jactatana* because the increase of mating length may reduce the time available for female feeding and oviposition (Keller and Reeve 1995) and increase predation risks (Rowe 1994, Bissondath and Wiklund 1996a).

For *C. jactatana* the courtship display length remains statistically similar for all matings but the activation time significantly increases with the increasing number of matings. This suggests that male mating history has little effect on the quality of the male courtship display and/or virgin females accepting mating attempts as long as males perform the courtship of similar length. It is also suggested that mated males need more time to start courtship or females need a longer call to stimulate mated males' courtship behaviour. Therefore, the impact of male multiple mating is two-folded. First, a longer call by females (pheromone release) for mated males can pose the former higher risks of being attacked by predators and parasitoids, and second, longer activation time in mated males may reduce their mating success as females prefer to mate with virgin males (Section 5.3).

Male mating history has significant effect on female reproductive fitness in *C. jactatana*. Like other moth species such as the tortricids *Z. canadensis* (Carroll, 1994) and *Choristoneura rosaceana* (Delisle and Bouchard 1995), *C. jactatana* males produce smaller spermatophores in successive matings and virgin females receiving spermatophores from mated males give lower fecundity and fertility. It is suggested that smaller spermatophores contain fewer sperm and less MDS, reducing

female reproductive fitness. In addition, *C. jactatana* females emerge with no mature eggs (Section 3.4.2.2), suggesting that MDS play an important role in production of eggs and somatic maintenance of females (Wiklund et al. 1993; Svensson et al. 1998).

Foster and Ayers (1996) report that *Epiphyas postvittana* females mating with mated males will have shorter refractory period and resume calling earlier than females mated to virgin males. Similar results were also obtained for *Cy. pomonella* (Howell et al. 1978) and *L. botrana* (Torres-Vila et al. 1997). However, the size of the first spermatophore a *C. jactatana* female receives has no effect on the female remating behaviour as reported in Section 4.3. It is suggested that whether or not the *C. jactatana* female remates is independent from the mating history of her mates.

Females mating with mated males have shorter oviposition periods than those mated to virgin males in *C. jactatana*. Gillott (2003) suggests that MDS induces ovulation, ova maturation and regular oviposition. The inadequate supply of sperm and MDS from multiply mated males could be responsible for the decline in oviposition length (Foster and Ayers 1996).

# CHAPTER 5
# MATING BEHAVIOUR AND SEXUAL SELECTION OF *CNEPHASIA JACTATANA*

## 5.1 General Introduction

Since Darwin (1859) highlighted the evolutionary importance of competition for fertilising the female ova, the study of mating behaviour has received massive attention. The mating sequences contain valuable information needed to understand insects' mating systems. Similarly, discovering the traits selected by males and females provides information necessary for the implementation of behavioural based control tactics. The first study detailed in this chapter describes the mating behaviour of *C. jactatana* and the tactics employed by males to compete for a virgin female. The second part of this chapter reports the attributes selected by males and females in relation to age, virginity and body size.

## 5.2 Mating Behaviour of *Cnephasia jactatana*

### 5.2.1 Introduction

The understanding of mating behaviour of a species is mandatory prior to the establishment of any behavioural control technique (Butt 1991). Most of our knowledge of the mating behaviour of tortricids relates to *Cydia pomonella* (Borden 1929, Gehring and Madsen 1963, Howell et al. 1978, Castrovillo and Cardé 1979 and 1980, Howell 1991), *Cy. fumirefana* (Sanders and Lucuik 1972 and 1992) and *G. molesta* (Gustan 1964, Baker and Cardé 1979a and 1979b). In general the tortricid mating behaviour involves several steps: a) females release sex pheromones, b) males detect the pheromonal blend and locate the female, c) males court females, and d) mating.

Stevens and McKenna (1999) suggested the development of mating disruption techniques to control *C. jactatana* in kiwifruit orchards. Section 4.2 showed that a technique that delayed mating would have an important impact on the

reproductive capacity of this species. However, no information on its mating behaviour is currently available. The aim of this section thus was to investigate the mating behaviour of *C. jactatana* by: a) observing the mating sequences of the virgin insects, b) comparing the different strategies used by virgin and mated males to achieve mating, and c) describing male-male interactions (intra-sex competition).

### 5.2.2 Materials and Methods

#### 5.2.2.1 Insects and Observation Arena

Insects were individually reared (Section 3.3.2.1). Pupae were sexed and weighed and kept individually in glass vials until emergence. Adults were kept individually in glass vials until the experimental day (Section 3.2.1). Males and females were placed in separate rooms. All experiments were conducted during the scotophase under a reverse-light regime (section 3.2.3).

I used test cylinders (Section 3.2.1) as observation arenas. No food or oviposition substrate was provided. Continuous light from red photographic safe lamps (Phillips No. B22PF712B, Made in EC) (e.g. Webster and Cardé 1982) was utilised to observe mating behaviour.

#### 5.2.2.2 Mating Behaviour

According to Section 3.4, *C. jactatana* females were not sexually receptive until they were $\geq$2 d old. Therefore, 3-d-old insects were used for experiments. Fifteen minutes before the beginning of the scotophase, I introduced into the arena a virgin female and a virgin male. I started recording behaviour 15 min into the scotophase and ended 30 min after mating occurred. I observed 32 pairs in this experiment.

In the second experiment, the mating behaviour of 35 triads formed by a virgin 3-d-old female, a virgin 4-d-old male and a once-mated 4-d-old male was observed. To differentiate between males, I marked the insects with a correction pen (Mitsubishi pencil Co., Ltd. Tokyo, Japan) on the dorsal thorax. The mark did not restrain their movement in any way and did not modify their chances to achieve mating (Binomial test, $P > 0.05$; Hollander and Wolfe 1999). The potential partner to

mark was selected randomly for each replicate. Males of similar pupal weight were used for this experiment. Behaviour recording was made following the methodology described above.

### 5.2.3 Statistical Analysis

Frequencies of the observed behaviours were tabulated in first-order (Markovian) contingency tables, and comprehensive ethograms were devised. Such tables gave the frequency of transition from one behavioural activity to all other possible activities. $\chi^2$ values of all cells with probabilities > 0 were calculated and transitions with frequencies significantly higher than the expected value were used in construction of ethograms (Fagen and Young 1978). When appropriate, the Mann-Whitney test, McNemar test, $\chi^2$ test (Hollander and Wolfe 1999), z test (Freedman et al. 1998) or *t* test was used to analyse different behaviours.

### 5.2.4 Results

#### 5.2.4.1 Mating Behavioural Sequences

After release into the arena, most insects remained motionless or walked randomly for the first 30-90 min into the scotophase. During this time males generally walked randomly on the floor, walls and ceiling of the arena. If males touched females, in most cases (86%) females would walked away; in 10% of encounters males would move away, and in 4% both insects remained motionless for a short period of time and then resumed walking. Some females walked up and down the wall of the container, shaking their body and vibrating their wings (< 5 s). Nevertheless, no expanded pheromone gland was observed and males did not modify their behaviour due to these female activities.

The sexually related activities (Figure 5.1) appeared to commence about 2 h into the scotophase. These activities started when the male walked towards the female in a zigzag pattern and positioned his head to the female's. In 40% occasions,

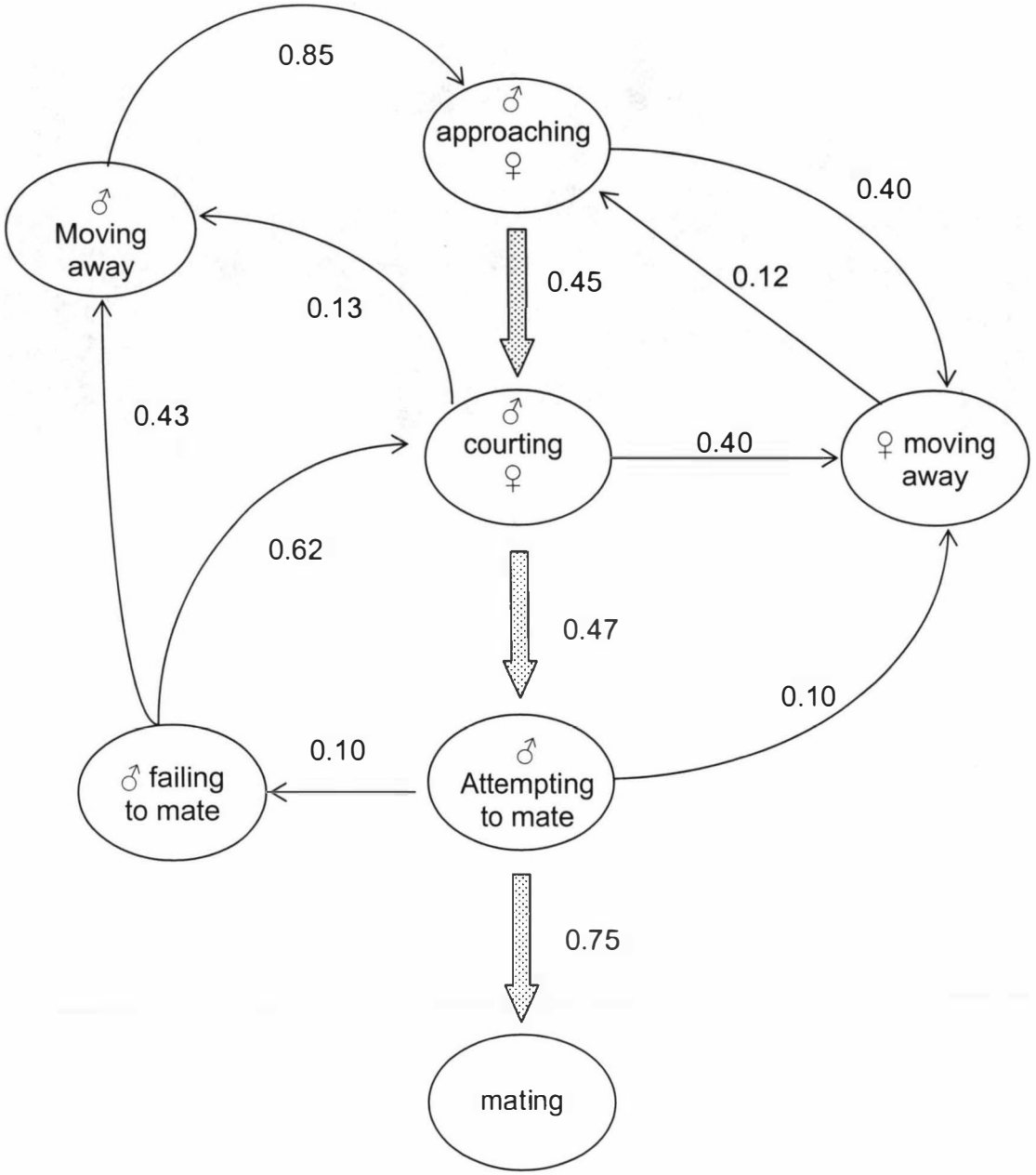


**Figure 5.1** Flow chart of the mating behavioural sequences of *Cnephasia jactatana*. Probabilities of a particular transition between stages are given. Only first-order transitions with frequencies higher than expected events ($P > 0.05$) are included. $n = 32$

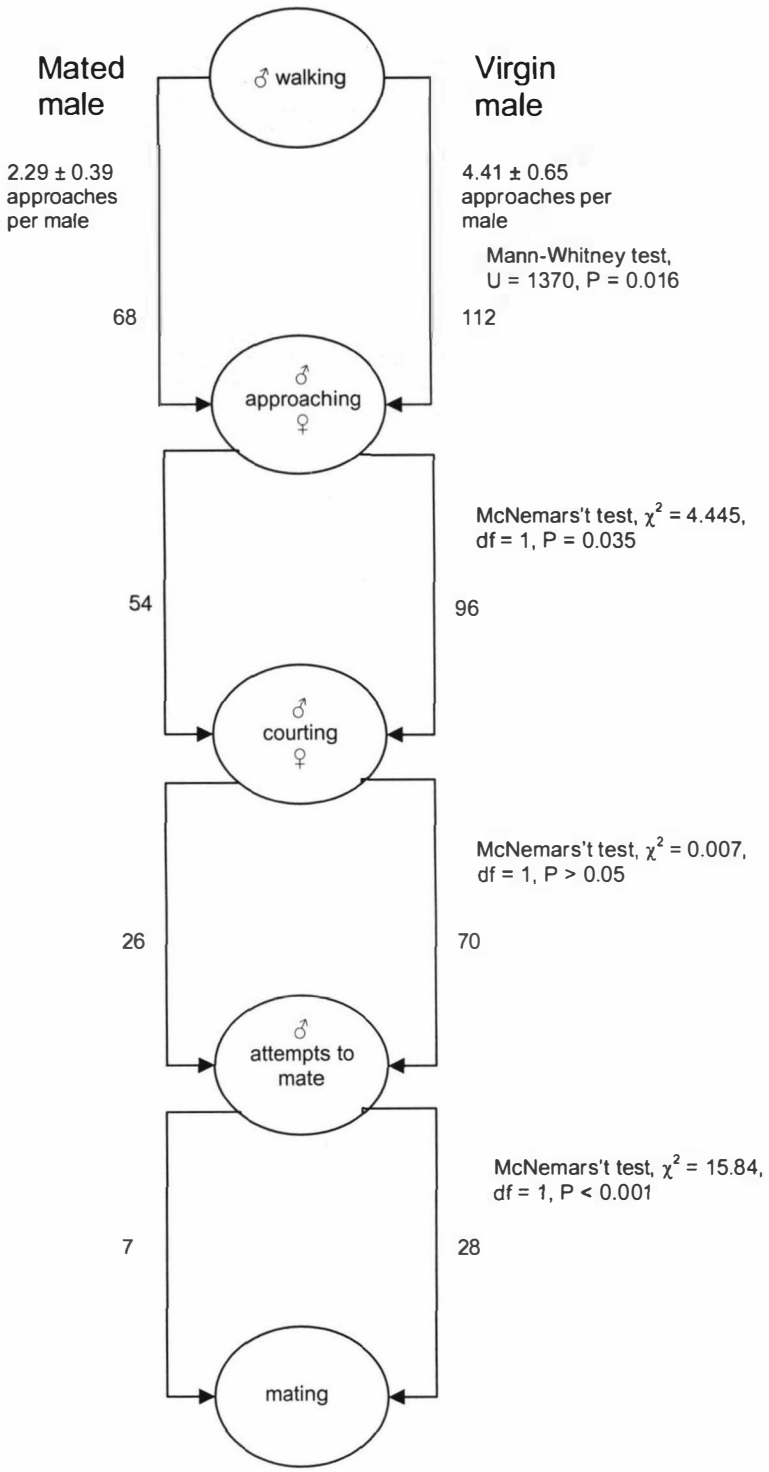


**Figure 5.2** Comparison of the number of attempts made by mated and virgin males when competing for a virgin female. The total occurrences of each behaviour among 35 triads are indicated in the figure.

the female moved away from the male. The rejected male remained motionless and resumed walking after a brief while. If the female accepted the male's presence in a head-to-head position, she would remain where she was and he would touch her head with the tips of his fanning wings (courtship display). After the courtship display occurred, in 13% chances the male would suddenly terminate it and moved away; in 40% chances the female would walk away; and in about half of the cases the male would constantly fan his wings, touch her abdomen with the tips of his wings, and attempt to mate. He raised his abdomen and exposed his claspers, and then he positioned himself beside her with both sexes facing the same direction. When this happened, in most cases the male reduced wing-fanning frequency and mating occurred but in 10% cases the male failed to mate with the female or the female moved away (10%) by walking, pushing the male away with their hind legs, or moving her abdomen. As shown in Figure 5.1, if mating failed to occur the male would repeat the above process. During mating, the insects faced opposite directions. After $\geq$1 h, mating ended and the male did not show any further interest in the female.

#### 5.2.4.2 Differential Mating Success in Virgin and Mated Males

In triad trials, very few activities occurred during the first 30 min of the scotophase. An increase in activity was observed about 1 h into the scotophase.

Both virgin and mated males followed the same pathway when approaching receptive females (Figure 5.1). However, virgin males approached females significantly more times than mated males (Figure 5.2). Similarly, virgin males performed significantly more courtship displays and were more likely to achieve mating than mated males (Figure 5.2). Virgin males approached and courted females significantly earlier than mated males (z test, $z = 3.27$, $P = 0.001$ for approaching female and $z = 3.77$, $P < 0.001$ for courting). Unsuccessful males (3) courted the recently mated females for a short period of time (< 10 s) but failed to mate.

Virgin males approached females significantly more times in the presence of rival males than in the absent of rival males (Table 5.1). However, the number of courtships and mating attempts remained similar with or without rival males (Table 5.1).

**Table 5.1** Comparison of the number of sexual activities (means ± SE) of a virgin *Cnephasia jactatana* male in the presence and absence of a rival mated male

| Behaviour | With rival male n = 35 | Without rival male n = 32 | *t* | P |
|---|---|---|---|---|
| Approach | 6.01 ± 0.58 | 4.12 ± 0.47 | 2.53 | 0.01 |
| Courtship | 2.57 ± 0.17 | 2.74 ± 0.49 | 0.33 | > 0.05 |
| Mating attempt | 2.0 ± 0.14 | 1.71 ± 0.21 | 1.16 | > 0.05 |

#### 5.2.4.3 Male-Male Interactions

In the triad trials I found obvious male-male interactions. If the female was courted by both males simultaneously, she would quickly walk away. In some cases (3) males showed homosexual behaviour by courting to each other but such behaviour usually lasted < 5 s. Mated and virgin males performed similar number of homosexual courtships (z test, z = 0.99, P > 0.05). Mating pairs were sometimes disrupted by the other male. Virgin males disturbed the mating couple (either by wing fanning or mounting over the mating pair) in 3 occasions. In one case, the virgin male succeeded in setting the couple apart and copulated with the female. No mated male was found to disrupt the mating couple.

### 5.2.5 Discussion

The mating sequences of *C. jactatana* show a fairly fixed pattern. Males played an active role in approaching, courting and attempting to mate while females remained sedentary. The relatively simple behaviour of *C. jactatana* resembles that of other tortricids like *Ch. fumiferana* (Sanders and Lucuik 1992) and *Eupoecilia ambiguella* (Schmieder-Wendel and Schruft 1990) but contrasts with that described for *Cy. pomonella* (Castrovillo and Cardé 1980) and *Cryptophlebia leucotreta* (Zagatti and Castel 1987) where more complex interactions between male and female are involved.

The calling female of *Cydia pomonella* raises her body from the substrate and the ovipositor is protruding from the abdomen (Castrovillo and Cardé 1979). The calling female in a closely related species, *G. molesta,* presents similar calling posture: wings elevated, legs extended and abdomen risen from the substrate and the

ovipositor's anal papillae extruded (Gustan 1964, Baker and Cardé 1979a). Sanders and Lucuik (1972) described a similar calling posture as described above for *Ch. fumiferana* except that females may or may not completely extrude the ovipositor when calling, making calling detection difficult. Other tortricid females like *E. postvittana* (Lawrence and Bartell 1972), *Platynota stultana* (Webster and Cardé 1982), *Cryptophlebia leucotreta* (Zagatti and Castel 1987) and *Strepsicrates macropetana* (Mauchline 2000) assume similar calling behaviour. However, none of the above descriptions mentioned the female body shaking and wing fanning as part of the female calling behaviour. Such behaviour observed in *C. jactatana* does not appear to modify males' behaviour. It is suggested that either this behaviour is not related to female calling or it is a calling behaviour but the *C. jactatana* female sex pheromone has little effect on male behaviour in short distance as the pheromone is used for long-range attraction (Arn 1991).

Courtship success in *C. jactatana* appears to be more dependent on the female than on the male. In most cases the female rejects the first courtship attempt by the male. Her response to his courtship is either moving away or remaining still, which may involve selection of some male traits. The female may reject a mating attempt at any stage including when the male has established contact with her. It is still not clear whether *C. jactatana* males or females have means to assess their partner's quality during this brief period of time. *Cy. fumiferana* females seem to assess male quality before mating and may also reject males at first contact or even during males' mating attempt (Sanders and Lucuik 1992).

Tactile stimulation may play an important role in the *C. jactatana* mating success. To assure the female's acceptance the male needs to be in a head-to-head position with her. This position allows him to contact her with his antennae and labial palps, which may have a role in sex recognition and quality assessment (e.g. Schmieder-Wendel and Schruft 1990, Sanders and Lucuik 1992). Some tortricid males have pheromone glands on the hind wings (modified scales) which produce a blend used during courtship (Zagatti and Castel 1987, Benz 1991). There may be similar scent scales on *C. jactatana* wings and the observed behaviour of the male's touching the female's head and abdomen with wings could function as the chemo-tactile stimulation.

The female courted by both virgin and mated males has the opportunity to assess males' quality and select the "better" one for mating. Virgin males invest more energy for courting females than mated males in *C. jactatana*. This effort resulted in more virgin males achieving mating than mated males, supporting Darwin's sexual selection theory (Andersson 1994). When the operational sex ratio is male-biased, males must increase their mating effort to achieve mating (Halliday 1983, Jennions and Petrie 1997) but virgin males usually have more energy reserves and thus are more likely to achieve mating.

Whether females release a short distance pheromone to signal or attract a male is unknown. It appears that behavioural or sound cues may be used at close range in identifying a potential mating partner (Svensson 1996, Trematerra and Pavan 1995). Visual cues are highly unlikely as *C. jactatana* insects mate in darkness (Bathon et al. 1991).

## 5.3 Sexual Selection of *Cnephasia jactatana* in Relation to Age, Virginity and Bodyweight

### 5.3.1 Introduction

The study of insect mating systems allows an understanding of the evolutionary mechanisms that have moulded the behaviour and morphology of the species (Thornhill and Alcock 1983, Wiklund and Forsberg 1991). Darwin (1859) suggested that females should be choosier than males in their selection of mates because eggs cost more to produce than sperm, females mate fewer times than males, and females endure most of the parental care. This leads to the conclusion that females have more to lose from suboptimal matings than males do. Therefore, males should suffer the effects of sexual selection (Alexander et al. 1997).

In this section I evaluated the role of the age, virginity and size in the mate choice for both sexes in *C. jactatana* Walker.

### 5.3.2 Materials and Methods

#### 5.3.2.1 Insects and Mating Arena

Insects were obtained from the individual rearing system (Section 3.3.2.1). Pupae were sexed and weighted and kept individually in glass vials until adult emergence to ensure virginity. They were divided into three groups according to their weight: light, average or heavy. A light or heavy pupa was defined as one whose weight was less or greater than one standard deviation from the mean weight (mean ± SD, 41.54 ± 7.78 mg and 28.0 ± 4.35 mg for the female and male, respectively). Male and female pupae were allocated into different rearing rooms. Adults were checked for emergence at 0900 h and were used only once for experiments. All experiments were conducted under a reverse-light regime (section 3.2.3).

For each replicate of the following experiments, I released insects into test cylinders (without oviposition substrate and food dispensers) at the beginning of the dark period and continuously observed their behaviour until mating occurred. Afterwards, mated females were dissected to verify the presence of a spermatophore in their bodies, and all moths were preserved in 70% alcohol for morphological

measurement. Potential partners were selected and marked as in Section 5.2.2. The mark did not restrain insect movement (Binomial test, $P > 0.05$; Freedman et al. 1998). Insects of average size were used in the first two of the following experiments.

#### 5.3.2.2 Influence of Age at Mating on Mate Selection

The objective of this experiment was to determine whether males and females discriminated between partners based on their age. According to Section 3.4, sexual maturation period in *C. jactatana* were 1 and 2 d for males and females, respectively. If females and males mated when 6-d-old their reproductive potential was reduced by $\approx 60$ and $\approx 30$ %, respectively, as compared to 3-d-old pairs (Section 4.2). Therefore, I considered a 3-d-old insect as young and a 6-d-old insect as old in this study. For each replicate, I released a young virgin moth (selector) and two virgin potential partners (young and old) to the arena and observed the selection behaviour for mates. I performed 56 replicates for female selectors and 28 replicates for male selectors (Table 5.2).

#### 5.3.2.3 Influence of Virginity on Mate Selection

This experiment was designed to test whether *C. jactatana* moths selected partners according to their mating history. Results from Section 4.3 indicate that females accepted a second mating 3 d after the first mating. Therefore, I released a 6-d-old virgin female and a 6-d-old mated female (mated at 3 d old) for a 3-d-old virgin male to choose for mating. A total of 16 triplets were used in this treatment (Table 5.3).

According to Section 4.4, *C. jactatana* males could deliver another spermatophore 24 h after the first mating. Thus, I released a 4-d-old virgin male and a 4-d-old mated male (mated the day before) for a 3-d-old virgin female to select for mating. I repeated this experiment 25 times (Table 5.3).

#### 5.3.2.4 Influence of Body Size on Mate Selection

The objective of this experiment was to determine whether males and females chose partners based on their bodymass and antennal/wing length. All insects used for this experiment were virgin and 3 d old. As a replicate, a virgin selector (male or female of a specific weight class) was caged in the test cylinder with three virgin potential mates (light-, average- and heavy-weight) and allowed to mate. A total of 69 males and 70 females were used as selectors (Table 5.4 and Table 5.5). After experiments, the left antennae and left front wings of all moths were measured under a dissecting microscope.

### 5.3.3 Statistical Analysis

To test whether insects selected their partners based on age or virginity, I used a binomial test. A general model (PROC GENMOD) followed by a least squared means test was used to analyse the influence of weight on mate selection (Stokes et al. 2000). An analysis of variance (ANOVA) followed by a least significant difference test was used to compare antennal length. The relationship between antennal length and front wing length was assessed by a linear regression and slopes were compared by an analysis of covariance (ANCOVA).

### 5.3.4 Results

#### 5.3.4.1 Influence of Age at Mating on Mate Selection

In the current study, males actively selected young over old females for mating but females did not discriminate between old and young males for mating (Table 5.2).

**Table 5.2** Effect of age on mate selection in *Cnephasia jactatana*

| Selectors | n | Selectees | | P |
|---|---|---|---|---|
| | | Young (3-d-old) | Old (6-d-old) | |
| Male | 28 | 22 | 6 | 0.0019 |
| Female | 56 | 27 | 29 | 0.4469 |

Binomial probability value under $H_0$: 0.5

### 5.3.4.2 Influence of Virginity on Mate Selection

Both sexes chose virgin partners significantly more than non-virgin ones for mating (Table 5.3) but males appeared to be choosier than females in selecting between virgin and mated partners for mating (z test, z = 1.89, P = 0.058; Freedman et al. 1998).

**Table 5.3** Effect of mating history on mate selection of *Cnephasia jactatana*

| Selectors | n | Selectees | | P |
|---|---|---|---|---|
| | | Virgin | Non-virgin | |
| Virgin Female | 25 | 18 | 7 | 0.0216 |
| Virgin Male | 16 | 16 | 0 | <0.001 |

Binomial probability value under $H_0$: 0.5

### 5.3.4.3 Influence of Body Size on Mate Selection

The results show that *C. jactatana* male body weight did not affect his probability of being chosen for mating ($\chi^2$ = 4.69, df = 4, P = 0.3205) (Table 5.4). However, males with longer antennae had significantly higher mating success than males with shorter antennae (ANOVA, F = 9.34, df = 1,199, P = 0.002) (Figure 5.3).

**Table 5.4** Effect of bodyweight on mate selection by *Cnephasia jactatana* females.

| Female weight class (selector) | Male weight class (selectees) | | | n |
|---|---|---|---|---|
| | Light | Average | Heavy | |
| Light | 9 | 4 | 12 | 25 |
| Average | 8 | 9 | 8 | 25 |
| Heavy | 7 | 8 | 5 | 20 |
| Total | 24 | 21 | 25 | 70 |

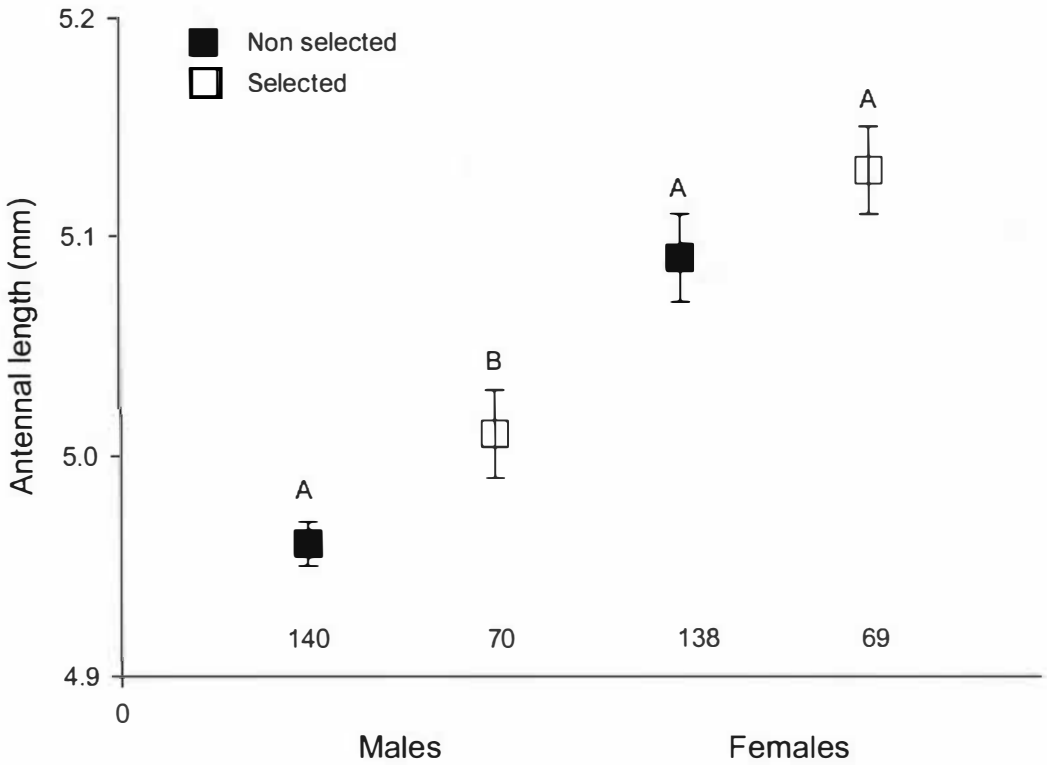


**Figure 5.3** Antennal length of selected and non-selected partners in *Cnephasia jactatana*. Bars with the same letter for each sex are not significantly different (ANOVA, $P > 0.05$). Sample sizes are on top of x axis.

The linear relationship between antennal length and front wing length between selected and non-selected males did not differ (ANCOVA, $F = 1.0$, $df = 1,197$, $P > 0.05$) (Figure 5.4).

The current study shows that *C. jactatana* males selected their mates based on both their own and their mates' body size ($\chi^2 = 12.48$, $df = 4$, $P = 0.0141$) (Table 5.5). Light males preferred light females while average males chose average and heavy females for mating. Heavy males did not have preference over female weight class when they selected a mate.

Antennal length (ANOVA, $F=1.61$, $df = 1,180$, $P > 0.05$) (Figure 5.3) and the linear relationship between antennal length and front wing length (Figure 5.4) did not differ between selected and not-selected females (ANCOVA, $F = 0.03$, $df = 1,180$, $P > 0.05$).

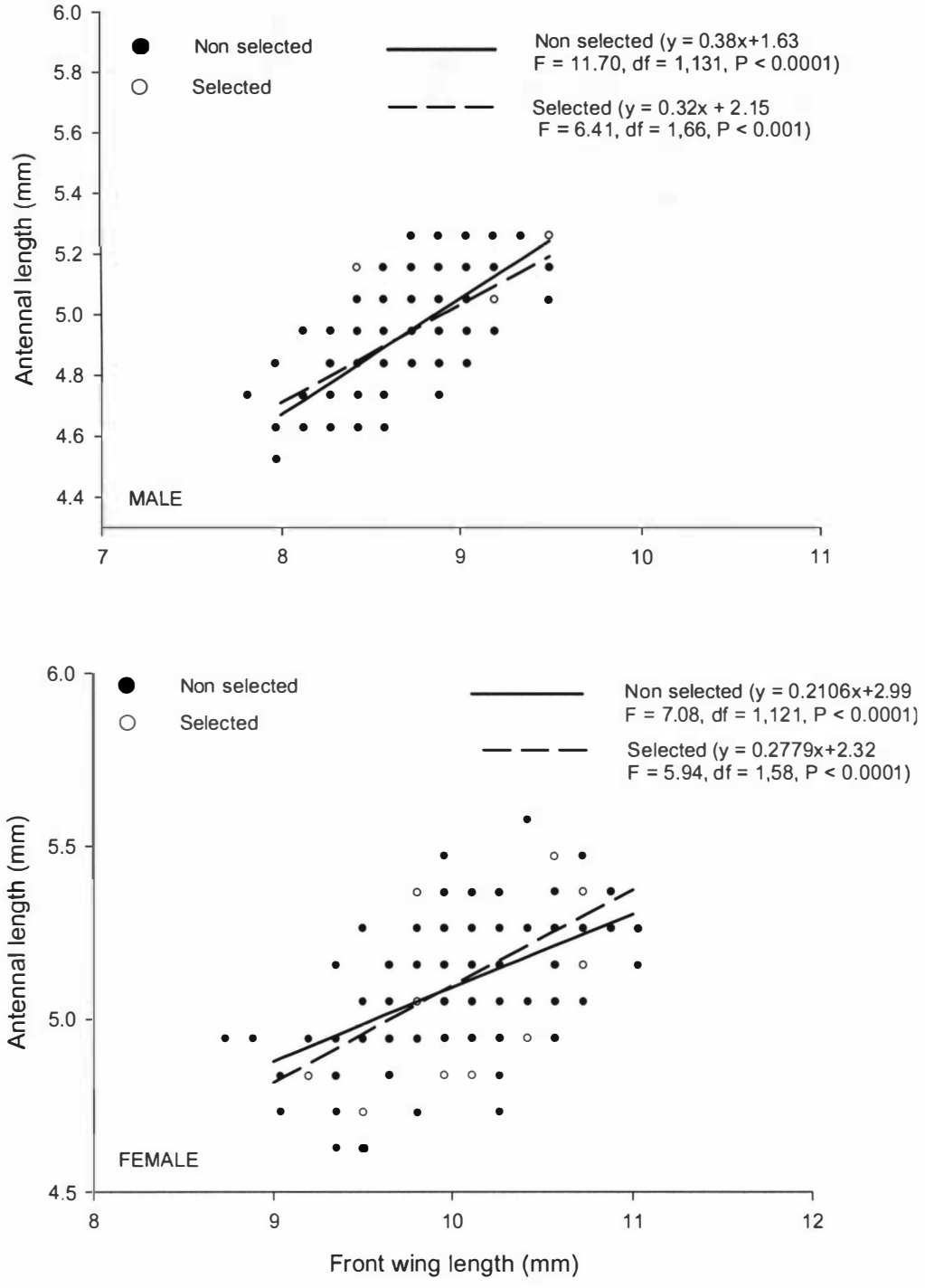


**Figure 5.4** Relationship between antennal length and front wing length in selected and non-selected males and females of *Cnephasia jactatana.*

**Table 5.5** Effect of bodyweight on mate selection by *Cnephasia jactatana* males

| Male weight class (selector) | Female weight class (selectees) | | | n |
|---|---|---|---|---|
| | Light | Average | Heavy | |
| Light | 12a | 6ab | 4b | 22 |
| Average | 2b | 12a | 9ab | 23 |
| Heavy | 7a | 8a | 9a | 24 |
| Total | 21 | 26 | 22 | 69 |

Figures in the same row followed by the same letter are not significantly different ($P > 0.05$).

### 5.3.5 Discussion

The fact that *C. jactatana* males preferred young females for mating supports the classical sexual selection theory. Females lose their scales as they age, and this may provide a mechanism for age discrimination by males over short distances (Cardé et al. 1989), either through mechanical or chemical stimulation (Grant et al. 1987). Section 4.2 demonstrated that the aging process severely affected females in terms of reproductive fitness. For example, if *C. jactatana* females mated when 6-d-old their reproductive potential decreased by more than 60% as compared to females that mated when 3-d-old. Therefore, investment in terms of sperm and male-derivate substances (MDS), in an old female, is highly risky, explaining the male's preference for young females for mating. The age related reduction in the female reproductive potential appears to be attributed to re-absorption of the ova or the use of resources entitled for reproduction for body maintenance (Lum 1979, Proshold et al. 1982).

However, the aging effect on *C. jactatana* male reproductive fitness is not as severe as on females. For example, relative to a 3-d-old virgin male, a female mating with a virgin 6-d-old male would reduce her fertility by only 27% (Section 4.2). Therefore, age selection by females for males is not as important as that by males for females. This reduction in male fertility may be attributed to a decline in sperm mobility and number (Hinds and Linley 1974).

Mating history in males and females has different consequences for both sexes and their potential partners. Mated *C. jactatana* females are receptive 3 d after the first mating (Section 4.3). By this time, mated females are 6 d old and have already laid ≈ 50% of their egg-load (Section 3.4). Therefore, by avoiding mated females and selecting virgin ones for mating, males reduce sperm competition and sire a larger number of offspring thus increasing males' fitness. It is suggested that males' selection of partners according to their virginity at least fits the "best of N-rule" (Svennsson 1996, Alexander et al. 1997). However, *C. jactatana* females' selection in this regard appears to match the 'threshold comparison rule' (Alexander et al. 1997, Jennions and Petrie 1997)

Males' discrimination between non-virgin and virgin females has also been reported by Cook and Gage (1995) for *Plodia interpunctella*, Wedell (1998) for *Coptaspis* sp. and Wedell and Cook (1999b) for *Pieris rapae*. In the last species, males assess the female mating status during copulation through their genitalia. They are able to detect the remains of spermatophores inside the females' body (Wedell and Cook 1999b). Whether *C. jactatana* can detect female's virginity during mating is unknown. Males may recognise females' mating history by short distant pheromone or sound (Svensson 1996).

Male body weight has no effect on mating success but males with longer antennae have higher mating success. Males with longer antennae may respond to chemical cues better and are advantageous in getting access to females. On the contrary, body weight rather than antennal length plays a major role in female mating success.

By mating with light females, light males suffer less sperm competition as light females generally do not remate (Section 4.3). More than 90% of average males preferred medium or heavy females for mating probably because they all have similar fecundity (Section 3.6). However, this weight-assortive mating process is not present in heavy males. A possible explanation of this behaviour is that generally heavy males produce larger ejaculates and are able to produce a second ejaculate earlier than light males (Carroll 1994). These characteristics confer heavy *C. jactatana* males a strategic advantage. They may mate with and compete for virgin females, as females do not discriminate between virgin and non-virgin males. In

addition, if large *C. jactatana* males show similar behaviour as *P. rapae* males, and do not ejaculate all available sperm in one mating, this enables them to tailor the number of sperm ejaculated in relation to the female mating history and their possibilities of achieving another mating (Wedell and Cook 1999b).

# CHAPTER 6
# GENERAL DISCUSSION AND CONCLUSION

## 6.1 Introduction

The paucity of knowledge on *C. jactatana* prior to this study provided an opportunity for me to explore its reproductive biology and mating behaviour. This thesis reports for the first time the reproductive potential, behaviour and sexual selection of *C. jactatana* and as such, gives an insight into the life story of this species.

In this chapter, I summarise and discuss main findings of my study and their relevance to the behavioural and evolutionary biology of *C. jactatana* and to the development of management measures for this pest.

## 6.2 Reproductive Potential and Population Growth

*Cnephasia jactatana* moths reared under food and density stress are lighter and less fecund with higher mortality rate compared to those reared under low or no such stress (Jimenez-Perez and Wang 2001). The lower performance of this species from poor rearing conditions may be attributed to faeces and microbial contamination (Stone and Sims 1992) and cannibalism between larvae (Singh 1977, Peters and Barbosa 1977).

The growth of the *C. jactatana* population is largely determined by the age of the female at the commencement of oviposition and the intensity of oviposition during the first few days of the oviposition period. The intrinsic rate of natural increase ($r_m$) is an excellent estimate for insect fitness as it combines in a single figure information on survival and biotic potential. However, different environmental conditions such as different kiwifruit varieties and cultural practices may produce different $r_m$ values (e.g. Leather and Dixon 1982). Therefore, studies similar to those of Cisneros and Barnes (1974) for *Laspeyresia (Cydia) pomonella* and Danthanarayana (1975a and 1983) for *E. posvittana* are mandatory to link different larval food with population growth.

Reproductive potential of this species is strongly related to bodyweight but this relationship differs between sexes. Males' reproductive fitness increases linearly with their increasing bodyweight. The linear relationship between bodyweight and reproductive fitness occurs in light and average females but disappears as female bodyweight reaches 50 mg. At this point, a further increase in females' bodyweight no longer increases their reproductive fitness. However, larger females have a shorter pre-oviposition period and higher daily fecundity. This may contribute to the increase of offspring survival and reduction of female pre-reproductive mortality, boosting population growth.

## 6.3 Reproductive Rhythms

Adult activities are closely correlated with age and circadian rhythms in *C. jactatana* (Jimenez-Perez et al. 2002). Males become sexually mature 1 d after emergence but females need a minimum of 2 d to become sexually receptive, coinciding with females' gonadic maturation period. Young moths prepare for reproduction by active feeding during the first 2 d of their life, ensuring energy reserve and gamete production and maturation.

In a 24-h cycle, reproductive activities occur almost exclusively in the scotophase, and are closely related temporally. Among these activities, oviposition always takes place earlier than mating in a given night, suggesting that few females lay eggs in the same scotophase as mating occurs. This may be due to the lengthy movement of sperm from bursa copulatrix to vestibulum where fertilisation occurs as in *Cy. pomonella* (Benz 1991). In addition, the short time between mating and the end of the scotophase may also deter oviposition until the next scotophase.

Age at the first mating is critical to insect reproductive fitness. Unlike many previous studies (e.g. Foster and Ayers 1996, Knight 1996, Vickers 1997, Knight and Turner 1999), we studied the subject by setting up pairs with a series of age combinations and thus quantify the differential effect of mating age on reproductive fitness of different sexes in *C. jactatana* (Jimenez-Perez and Wang 2003). We demonstrated that reproductive fitness is significantly affected by mating age of both sexes and delaying mating for 2–3 d severely reduces the reproductive potential of *C. jactatana*. We have also found that females are more severely affected by mating

delay than males in terms of reproductive fitness. Therefore, the phenomenon of males emerging earlier than females can be considered a strategy to maximise the probability of mating at an optimal age in this species.

## 6.4 Multiple Mating and Reproduction Fitness

Multiple mating increases reproductive fitness of both sexes in *C. jactatana.* However, energy reserves from immature stages of both sexes determine whether remating occurs and how it affects reproductive fitness (Jimenez-Perez et al. 2003).

The increase of reproductive fitness in *C. jactatana* females due to remating may be attributed to receiving more sperm and male-derivate substances (MDS), which contribute to higher fertility and fecundity. Such increase in males is relevant to fertilising more eggs from diverse females. Despite the fact that light pairs benefit more from remating than heavy ones, the former remate less frequently than the latter. This may be explained as that (1) males developed under food stress in their immature stages may have more difficulties to accumulate enough reserves to produce a second ejaculate, and (2) heavy females are able to produce more eggs and thus need to obtain an extra supply of sperm and MDS to achieve their maximal reproductive gains.

*Cnephasia jactatana* females remating with virgin males have higher daily fecundity and shorter oviposition period, suggesting that ejaculates from virgin males can accelerate egg production and maturation. Fertility rate of females having continuous access to virgin males does not decrease with time whether or not remating occurs. This suggests that females have control over whether a remating is necessary to maximise her fitness. In addition, neither mating length nor spermatophore size affects female remating behaviour in *C. jactatana.*

Most *C. jactatana* males can mate 4 times and a few can achieve up to 6 matings as long as they have a minimum of 24-h recovering period but their fecundate capacity diminishes with the increasing number of matings. For example, virgin females receiving ejaculates from mated males have lower reproductive output than those mating with virgin males. It is suggested that spermatophores from mated males contain fewer sperm and less MDS, reducing the female reproductive fitness.

Regardless of their mating history, *C. jactatana* males have similar courtship display and mating periods for all matings. This behaviour is strategically important for *C. jactatana* because the increase of mating length may reduce the time available for female feeding and oviposition (Keller and Reeve 1995) and increase predation risk (Rowe 1994, Bissondath and Wiklund 1996a). However, mated males need more time to start courtship or females need longer calls to stimulate mated males' courtship behaviour.

## 6.5 Mating Behaviour and Sexual Selection

The relatively simple mating behaviour of *C. jactatana* resembles that of *Cy. fumiferana* (Sanders and Lucuik 1992) and *Eupoecilia ambiguella* (Schmieder-Wendel and Schruft 1990) but differs from that of *Cy. pomonella* (Castrovillo and Cardé 1980) and *Cryptophlebia leucotreta* (Zagatti and Castel 1987) where more complex interactions between male and female are involved.

*Cnephasia jactatana* males appear to play a more active role in mating behaviour involving approaching, courting and attempting to mate. Courtship display by the male involves wing fanning, and antennal, palpal and wing contact with the female. Antennal and palpal contact may have a role in sex recognition and quality assessment as in other tortricids (e.g. Schmieder-Wendel and Schruft 1990, Sanders and Lucuik 1992). Some tortricid males have pheromone glands on the hind wings, which produce a blend used during courtship (Zagatti and Castel 1987, Benz 1991). There may be similar glands on *C. jactatana* wings and the observed behaviour of the male's touching the female with wings could function as chemo-tactile stimulation. Females appear to prefer virgin to non-virgin males for mating when given a choice. This may be because virgin males invest more energy for courting females than mated males in *C. jactatana*.

The calling behaviour in other tortricid females (raising body from the substrate and protruding ovipositor) does not occur in *C. jactatana*. Females of *C. jactatana* are observed to shake their body and fan their wings. However, such behaviour does not appear to modify males' behaviour. Therefore, details of *C. jactatana* female calling behaviour are worth further exploring. Whether females release a short-range pheromone to signal or attract a male is unknown. Behavioural

or sound cues may be used at a close range in identifying a potential mating partner in *C. jactatana* as in other moths (Svensson 1996, Trematerra and Pavan 1995).

The study of the cues used by *C. jactatana* adults to differentiate potential partners may provide a better understanding of the mating system of this species and lead to the development of measures for mating disruption useful for pest management.

*Cnephasia jactatana* mating behaviour does not completely fit Darwin's (1859) view of sexual selection where young, virgin and large partners are preferred for mating and females are more selective than males in mate selection. In *C. jactatana* male age has little effect on female's choice of mates. This may be because aging effect on *C. jactatana* male reproductive fitness is not as severe as on females (Jimenez-Perez and Wang 2003). Male bodyweight also has no effect on mating success but males with longer antennae are more likely to be chosen by females. It is suggested that such males may respond to chemical cues better and are advantageous in getting access to females. Females also prefer virgin to non-virgin males for mating.

Males appear to be more selective than females as males choose their partners based on their age, virginity and bodyweight. *C. jactatana* males prefer young and virgin females for mating because the aging process severely affects females in terms of reproductive fitness and mating with virgin females reduces sperm competition. Light males prefer light females and average males choose average or heavy females for mating, and heavy males did not have any preference for body weight.

## 6.6 Conclusion

In this thesis I have reported and discussed my main findings of the reproductive potential and behaviour, and factors affecting the reproductive fitness for a New Zealand native tortricid moth *C. jactatana*. The work has provided a much firmer basis of knowledge of this pest than existed hitherto, and a more rounded perspective of the reproductive biology in the species. Such knowledge is vital, as noted in the thesis, to appraising prospects for further investigation of the reproductive biology in the field and integrated management of this increasingly important pest.

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# CERTIFICATE OF REGULATORY COMPLIANCE

This is to certify that the research carried out in the Doctoral thesis entitled "Reproductive Behaviour of *Cnephasia jactatana* Walter (Lepidoptera: Tortricidae)" in the Institute of Natural Resources at Massey University, New Zealand:

(a) is the original work of the candidate, except as indicated by appropriate attribution in the text and/or in the acknowledgements;
(b) that the text, excluding appendices/annexes, does not exceed 100,000 words;
(c) all the ethical requirements applicable to this study have been complied with as required by Massey University, other organizations and/or committees which had a particular association with this study, and relevant legislation.

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PhD. Candidate
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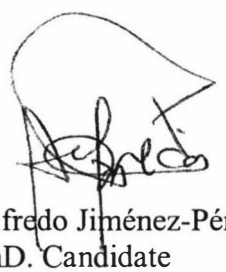
# CANDITATE'S DECLARATION

This is to certify that the research carried out for my Doctoral thesis entitled "Reproductive Behaviour of *Cnephasia jactatana* Walker (Lepidoptera: Tortricidae)" is my own work and that the thesis material has not been used in part or in whole for any other qualification.



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# SUPERVISOR'S DECLARATION

This is to certify that the research carried out for the Doctoral thesis entitled "Reproductive Behaviour of *Cnephasia jactatana* Walker (Lepidoptera: Tortricidae)" was done by Alfredo Jiménez-Pérez in the Institute of Natural Resources, Massey University, Palmerston North, New Zealand. The thesis material has not been used in part or in whole for any other qualification, and I confirm that the candidate has pursued the course of study in accordance with the requirements of the Massey University regulations.

Dr. Qiao Wang
11 Sep. 03