ASPECTS OF THE LIFE CYCLE, BIOLOGICAL PERFORMANCE AND

QUALITY OF THE BLACK LYRE LEAFROLLER

'Cnephasia' jactatana (Walker)

J. P. OCHIENG'- ODERO

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ABSTRACT

The thesis answers the general question of whether the quality of artificially reared insect species should be based on performance tests for intended use or whether quality should be based on a more holistic biological approach. The empirical research is carried out using the lepidopteran leafroller *'Cnephasia' jactatana* (Walker).

The thesis defines biological performance and quality in terms of the success of an insect population in survival and reproduction and regards the laboratory environment as an artificial habitat that insects must colonise in order to survive and reproduce. Changes in biological performance that occurred during 12 successive generations of laboratory rearing were due to selection, acclimatisation and domestication and not adaptation. Artificial colonisation is theoretically successful within a limited range of environmental factors. As the inherent genetic variability of the founder population determines the resilience of the population to changes in performance, the ranges of environmental factors during colonisation should be wide to 'capture' much of the variability.

Using body size (weight) as an aspect of overall quality, the thesis presents evidence that the final instar larva of *C. jactatana* has a threshold mechanism (larval critical weight, L_{CW}) that determines pupal and adult size. There is a proportionate

decrease in weight from the maximum weight that a larva attains in the final instar (L_{MW}) to pupa (described as constant D_P) and to adult (D_A). There is a direct relation between the latent feeding period (period between attaining an L_{CW} and L_{MW}), L_{MW}, pupal and adult size, and the reproductive performance (fecundity). Within the experimental conditions diet quality, temperature, photoperiod and artificial selection had no effect on the larval critical weight, D_P or D_A, the larval threshold mechanism in *C. jactatana* is probably a mechanically trigger that initiates pupation. Diet quality, temperature and thermophotoperiods affected pupal size, adult size and reproductive performance. Photoperiod had no significant effects on size and reproductive performance. Positive assortative selections for slow development and low pupal weight significantly decreased pupal and adult size, and reproductive performance. Selection for fast development and heavy pupal weight for three generations had no significant effect on size or reproductive performance. Larval critical weight is demonstrated as useful to define quality indices and predict the performance of laboratory reared insects.

The general conclusion of the thesis is that insect quality should be defined more in terms of the success in survival and colonising ability rather than solely on the success for 'intended role' or 'fitness for use'.

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A_{CW} Adult critical weight

A_W Adult weight

D_A Decrease in weight from larval maximum weight to adult

D_P Decrease in weight from larval maximum weight to pupa

GPD General purpose diet

L_{CW} Larval critical weight

L_{MW} Larval maximum weight

P_{CW} Pupal critical weight

P_R Pupation rate index

P_W Pupal weight

S_A Adult synchronism index

SBD Sheep nut bean-based diet

S_P Pupal synchronism index

CHAPTER / 1

GENERAL INTRODUCTION

1.1 Biological performance and the problem of insect quality

Rearing of insects under captivity and in artificial environments (laboratories, mass-rearing facilities, glass- houses) has been practiced for thousands of years (especially silkworm rearing and beekeeping, Singh and Ashby, 1985) but gained momentum after 1960 with the emphasis of biological methods as a basis for pest control. Development of artificial rearing methods has been responsible for the advance of many aspects of theoretical, basic and applied entomology (Knipling, 1966; 1984).

Use of artificially reared insects has sometimes run into problems of quality. Two examples will suffice to illustrate this point. The screwworm, *cochliomyia hominivorax* (Coquerel) was a serious pest to the livestock industry in Southwestern United States (Bush, 1976). In 1962, sexually sterile adult screwworm flies were released to suppress the the pest population. Within a year, there was a dramatic drop in wild populations. Releases of sterile adults continued at the rate of over 1 billion flies/year. By 1972, however, there was a resurgence of the pest despite a release five times more sterile flies than in 1962. Investigation into artificially reared flies showed that males mated less frequently

(Alley and Hightower, 1966) and less aggressively (Spates and Hightower, 1967). Observed changes in flight and mating behaviour of irradiated males were linked to genetic changes controlling the enzyme α-glycerophosphate dehydrogenase (Bush and Neck, 1976; Bush et al. 1976).

The second example is more recent. Agee (1986) found that the compound eyes of boll weevils (*Anthonomus grandis grandis* Boheman) artificially reared for many generations on artificial diets had reduced visual sensitivity than field-collected ones. The laboratory reared weevils, however, were being used for large-scale behaviour studies in South Carolina. The validity and usefulness of the results for sterile insect control could be therefore doubtful.

Research into quality aspects of artificially reared insects is common in most modern rearing facilities (Moore *et al.* 1985). Aspects of quality studied include flight ability, olfaction, pupal size, startle reaction, mating propensity (Boller and Chambers, 1977a; 1977b; Boller *et al.* 1981); pupal abdominal rotation, pupal weight and buoyancy, wingbeat frequency (Fisher, 1983); visual sensitivity (Agee 1977; 1986); Life cycle parameters (Brewer, 1983); pupation environment (Hooper, 1987). Boller *et al.*, (1981) introduced a standardized package of 5 tests (RAPID) to detect quality changes of mass-reared fruit flies, *Ceratitis capitata* while Fisher (1983) proposed a model for the quality control of

mass reared Lepidoptera. Boller *et al.* (1981), Fisher (1983) and Chambers and Ashley (1984) have demonstrated the use of industrial process control charts in the analysis of results obtained from the various quality tests.

Most of the research on the quality of artificially reared insects has only dealt with measurement or monitoring of specific traits of the overall quality of mass-reared insects. This trend has had the effect of insect quality being defined by the objective for rearing ('how well it functions in its intended role' Boller, 1972; 1979; Huettel, 1976; Bush, 1979 'Fitness for use' Chambers, 1975; 1977; 1980; Moore *et al.* 1985). In other words, depending on the end use of the insect the quality standards will be different. Huettel (1976) detected the confusion raised by this approach and therefore categorized insect quality into two types. The first type is related to the success of establishment of the laboratory population in the artificial environment and how well the laboratory insects perform in the field when released. The second type of quality is derived from the comparison of a specific trait or set of traits between the laboratory and the wild insect. The wild insect is adopted as a standard of comparison. Chambers (1977) described the definition of quality as existing 'only in the eye of the beholder' and redefined insect quality as biological skills relative to a standard. Boller (1979) divided overall quality into five major components (adaptability, motility, orientation, sexual activities

and reproduction) consisting of various biological aspects that were compared with the wild. Despite attempts to define insect quality, the literature portrays no clear understanding of what it actually means. Changes in the biological performance of laboratory population have been described as adaptations, some positive (improving the quality) and others negative. Hence, higher fecundity recorded after a number of generations is taken as evidence of how well the insect has adapted to the new environment and increase in pupal weight as evidence of the improvement in insect quality (see Leppla *et al.* 1976; 1980, 1983, Loukas *et al.* 1985 and Al-izzi, 1987). To date, most research effort is still targeted at measuring the magnitude of change in biological performance rather than how the changes relate to the performance of the organism as a biological entity. There is also the danger of research into insect quality segregating into a body of statistics that has no relation to natural life.

This thesis approaches the question of insect performance and quality differently. Insects are organisms designed to maximise and exploit the available resources (biotic and abiotic) to their advantage. All insects, irrespective of the habitats they live in have only one 'intended role', which is to maximise their survival and colonising ability (reproductive fitness). As such, there is only one type of biological performance and therefore quality, that is the success of the individual insect in survival and contribution

to the next generation. An insect that survives and contributes more to its survival and species range is of a higher quality than one that does not. Biological performance can be defined by how well the organism has contributed to its survival and reproduction in a given ecological system. Overall quality is the sum effect of all the attributes that make an insect population capable in surviving and enhancing it's colonising ability.

The thesis considers the laboratory and wild situations as two different ecological habitats, one natural the other artificial (see Mason *et al.* 1987). Insects approach the two systems with a similar purpose, that of survival and maximising their species range. Any difference between the two populations (wild and artificially reared) is regarded as due to different ecological demands and effect of the habitats (see also Robertson, 1965 on analysis of population differences).

As body size has been shown to be an integral part of an insect's performance (see for example Gunn and Gatehouse, 1986; Wagner *et al.* 1987), the thesis investigates body size (weight) under those aspects of physiology and development susceptible to environmental variation and how they relate to the reproductive performance of the insect. The interpretation of the results acts as an indicator of how overall quality of an insect may be affected by biotic and abiotic factors in the new ecological

habitat.

The thesis addresses the general question of biological performance and quality with the following objectives:

a) To infer changes in insect performance brought about by artificial colonisation and subsequent rearing for successive generations. To determine if the changes that occur in biological performance are true adaptions (Darwinian).

b) To test for the existence of a critical weight as a threshold for pupation. To establish relationships between critical weight, pupal and adult weight, latent feeding period and reproductive performance.

c) To investigate the effect of diet quality on the critical weight and reproductive performance

d) To investigate the effect of temperature on the critical weight and reproductive performance.

e) To investigate the effect of photoperiod and thermophotoperiod on the critical weight and

reproductive performance.

f) To evaluate the effect of assortative selections on
the critical weight and reproductive performance.

1.2 The insect

'Cnephasia' jactatana (Walker) (Lepidoptera: Tortricidae)
is the most widely distributed species out of the nine New Zealand
moths attributed erroneously to the genus *Cnephasia* (Hudson, 1928;
Dugdale, in prep.). The adult is a small moth with a wing span of
about 2cm. The forewing is a shade of brown with the female usually
lighter than the male (Hudson, 1928; Manson, 1965; Gaskin, 1966).
The most conspicuous characters are:

1) The thick wavy black streak near the base of each
forewing. When at rest, the two black streaks meet and tend to
resemble a jagged "S" on it's side (Gaskin, 1966) or a lyre
(Hudson, 1928; Manson, 1965).

2) The blackened labine paepi, fore cosae and underside of
the head (J. Dugdale, pers. comm.).

The larger larvae are light brown with a reddish hue. The
head capsule is light brown to golden in colour. The moth has been
referred to as 'The hook-marked bell moth' (Gaskin, 1966); 'The
lyre moth' (Manson, 1965) and more recently Steven (in press)

suggested the name 'The black lyre leafroller'.

1.2.1 Classification

Until recently the classification of tortricids was in some
confusion due to homoplasy (Horak, 1984 ; Horak and Common, 1985)
and character spread. The early concepts of nomenclature based on
external morphology (wing venation, wing shape) and the presence of
a series of plesiomorphies caused workers to assign this species to
the Palaearctic genus *Cnephasia* (Dugdale, 1966 and in prep.; Horak,
1984;). The recent advent of nomenclature based on genetilic
morphology (Obraztsov, 1954) and pheromone components (Roelofs and
Brown, 1982; Tamaki, 1985) has gone some way in sorting out the
confusion in tortricid classification. Genitalic morphology in *C.*
jactatana shows certain characteristics that do not fit into
Cnephasia (the uncus is not covered in setulae, there is no
characteristic transverse cleft in the ovipositor and the signum is
complete and daggered; Razowski, 1965). The musculature indicates
that the M_2 muscle is split and the aedeagus is unusually positioned
in copulation (Kuznetsov and Sterkolnikov, 1984; Dugdale, in
prep.). Pheromonal analyses have shown that *C. jactatana* posses
(Z) - 11 - tetradecenyl acetate (Z11-14:OAc) as one of the
functional attractant components and not the expected Z9-12:OAc
which Roelofs and Brown (1982) indicated as an attractant for
members of the palaearctic genus *Cnephasia* (e.g. *C. pumicana*
(Zeller), *C. stephensiana* (Doubleday), Tamaki, 1985; E.

Priesner, pers. comm.). Recent collections by J. Dugdale in the Western Pacific have led to some consideration of relationships based on *Sparganothis* characters and Dugdale proposes the name *Xenectis* for *jactatana* and sees a possible relationship with sparganothines, and species of *Xenotrictis* Meyrick (similarly polyphagous and arboreal) found in Fiji, Vanuatu and New Caledonia.

1.2.2 Habit and host range

Manson (1965) noted that *C. jactatana* had a varied habitat range, while Gaskin (1966) reported it feeding on *Eucalyptus spp.* Hudson in 1928 recorded the moth frequently inhabiting forests, being almost inconspicuous when on the ground or amongst dead leaves. *Citrus spp., Crataegus spp., Diospyros kaki, Eucalyptus spp., Fuchsia spp., Griselinia spp., Leptospermum spp.*, and *Phormium tenax* have been recorded as host plants of the moth (Spiller *et al.* 1982). On *Coprosma spp.* the larvae cuts one apical leaf, ties the dying leaf to a living one and feeds on the dying leaf tip. It appears *C. jactatana* may well be a secondary feeder, utilising mainly the dying or necrotic leaf material rather than live tissue (C. Green, J. Dugdale, pers. comm.).

1.2.3 Economic importance

In recent times, *C. jactatana* has been repeatedly reported on nearly all commercially grown horticultural crops although more often on Kiwifruit (*Actinidia chinensis* Planch until recently, now known

as *Actinidia deliciosa* (Chevalier), Sale 1980; 1983; Anon. 1985; Steven, 1988). Kiwifruit is a target of various diseases and pests, which are of concern due to stringent export standards. The leafroller species complex is, however, the most serious. These include the brownheaded leafroller *(Ctenopseustis obliquana* (Walker), the greenheaded leafroller *(Planotortrix excessana* (Walker), the lightbrown apple moth, *(Epiphyas postivittana* (Walker), *Epalxiphora axenana* (Meyrick) and *'Cnephasia' jactatana* (Walker) (Sale, 1983; Steven, in press). Steven (pers. comm.) now considers the importance of *C. jactatana* on Kiwifruit to be the third most important leafroller pest after *E. postivittana* and *C. obliquana*.

Larvae of most of the leafroller moths are primary leaf feeders, causing scarring on the surface by their feeding activity. Damage very early in fruit formation results in deformity and fruit drop. The latter, although more superficial, does not attract the local market, and are not permitted as export fruit (Ferguson, 1980; Sale, 1983). Kiwifruit vines that are not sprayed can suffer as much as 50-60% leafroller damage according to Sale and Steven (1984). What role exactly *C. jactatana* plays within the complex is still unclear (Steven, pers. comm.), but Wearing *et al.* (1980) note that the insect assumes importance at kiwifruit harvest, probably an indication of its secondary feeding habit. Steven (unpub.) records *C. jactatana* as the only leafroller likely to burrow deep into fruit,

a special concern in view of quarantine restrictions.

1.3 Style of presentation of the thesis

The thesis is presented as a series of chapters prepared as autonomous papers submitted to various international scientific journals for publication. The references have, however, been brought together at the end of the thesis to avoid repetition of common sources of literature. The 'General introduction' and 'General conclusion' chapters are used to bind the autonomous chapters into the general question of biological performance and quality of artificially reared insects. An appendix is included that contains information on the technique and equipment used to colonise and manage the laboratory population of *C. jactatana*.

CHAPTER / 2

LIFE CYCLE CHANGES IN 12 SUCCESSIVE GENERATIONS OF LABORATORY COLONISATION OF THE LEAFROLLER *'Cnephasia' jactatana* (WALKER) (LEPIDOPTERA: TORTRICIDAE) ON ARTIFICIAL DIET

2.1 ABSTRACT

Close monitoring of laboratory colonisation and successive rearing of the lepidopteran leafroller *'Cnephasia' jactatana* on artificial diet revealed no distinct impact on the life cycle. The second laboratory generation had prolonged development time and different sex synchronism in pupation and eclosion patterns. Most deleterious changes observed in latter generations (decrease in fertility, egg hatch, sperm motility; failure for mating adults to separate, pupal and adult malformations) were due to incompatibility to the general purpose diet (GPD) and were absent under sub-colonisation on the sheep nut-bean based diet (SBD). Success in the laboratory colonisation of *C.jactatana* is attributed to a random mating protocol, choice of environmental variables and a rapid rate of population growth. Rather than a true adaptive process, laboratory colonisation is proposed to depend on the plasticity of the tolerance limits in insect species. Insects will establish successfully in captivity if the magnitude of environmental variables are within the insects' ranges of colonisation. Optimum

physiological and behavioral responses are obtained when the

variables are within the smaller preferred range of colonisation.

Other concomitant processes at colonisation are selection,

acclimatisation and domestication. Definition of the terms

'adaptation' 'acclimatisation' and 'domestication' commonly misused

in describing processes during laboratory colonisation are proposed.

2.2 INTRODUCTION

Colonisation of insects from natural populations into

artificial habitats (e.g. laboratory, Mason *et al.* 1987),

maintained under simulated environments (e.g. constant temperature

and humidity, artificial illumination, sudden changing photoperiods)

is the basis of mass-rearing operations. The quality of insects

produced from such artificial habitats has assumed major importance

in recent years as pest control strategies have shifted emphasis to

biological and autocidal control methods (e.g. mate disruption,

augmentation of natural enemies, sterile insect release technique;

Boller and Chambers, 1977; Knipling, 1982; 1984; Curtis, 1985). The

success of these methods require considerable interaction between

wild populations and the artificially colonised insects often reared

in the laboratory for several generations.

Bartlett (1984a, b) noted that failure by insects reared

in captivity to function in the wild is mostly due to pathogenic

contamination or genetic deterioration. While pathogenic conditions are easily identified and amended, genetic problems are harder to identify and more difficult to rectify (Loukas *et al.* 1985; Mason *et al.* 1987). Change in genetic variability or heterozygosity is the main cause identified for deterioration of biological performance of laboratory reared insects (Nei *et al.* 1975; Joslyn, 1984; Moore, 1984;). Changes to the heterozygosity may be due to random events like genetic drift and founder effect (Berry, 1982) or directional events like inbreeding and Wahlund's effect (Joslyn, 1984). Broad variation of genetically controlled traits is important as it enables the traumatic change from a natural to an artificial habitat to occur successfully and with ease (Bush, 1975).

Monitoring of insect life cycle and behaviour has been suggested by Brewer (1983); Bartlett (1984a, 1985); Loukas *et al.* (1985) and Bernado *et al.* (1986) as a measure of changes due to alteration in the inherent genetic variability that may impede biological performance. Life cycle measurements are easy to determine, precise, indicative of changes and mortality at specific facets of insect development (Leppla *et al.* 1983). Boller (1972) identified potential changes at the reproductive stage as altered mating behaviour, changes in the premating and the postmating mechanisms, sperm mortality, hybrid inviability and hybrid breakdown. Other changes attributed to laboratory colonisation include loss of host plant specificity (Schoonhoven, 1967); decrease in pheromone

production (Gast, 1968); flight incapacitation (Rai, 1969; Bush and Neck, 1976); changes in pre-oviposition periods (Rössler, 1975) and visual impairment (Agee, 1986).

The term 'adaptation' has been frequently used to describe the processes that occur during the laboratory colonisation of an insect species (Leppla *et al.* 1976, 1980, 1983; Mackauer, 1980; Bartlett, 1984a, b; Loukas *et al.* 1985; Smith and Morton, 1985; Roush, 1986; Al-izzi, 1987 and Moore, 1987). 'Domestication' and 'acclimatisation' are two other terms that have been used to describe changes which occur during colonisation (Bartlett 1984 a, b). Boller (1972) described a 'production curve' for laboratory reared insects in which he predicted low reproductive performance in the first five generations with recovery in latter generations. A similar phenomenon has been described by Proshold and Bartell (1972) and Raulston (1975). Leppla *et al.* (1980) described changes at colonisation as laboratory adaptations which were parabolic (or non-linear, Leppla *et al.* 1983) characterized by rapid decline until the fourth generation (G_4) and total recovery by G_6.

Over a period of three years the Black lyre leafroller *'Cnephasia' jactatana* (Lepidoptera: Tortricidae) was reared under a random mating system (see Figure 2.1) for 12 successive generations. *C. jactatana* is currently ranked third in importance among the leafroller complex found on Kiwifruit in New Zealand

(Steven, 1988). The purpose of this experiment was:-

1) To identify progressive life cycle changes that occurred during laboratory colonisation of an insect species.

2) To evaluate whether the life cycle changes were adaptive.

3) To test for the 'production curve' under a system of random mating and under sub-colonisation on a different artificial diet.

2.3 MATERIALS AND METHODS

2.3.1 Laboratory rearing of the stock population

The insects used in this experiment were derived from mated female moths light trapped in the North Island of New Zealand which laid eggs that hatched to give 529 neonate larvae (G_1). The diet used during the establishment and rearing of the stock population was general purpose diet (GPD, Singh, 1983; see appendix Table A.4 for composition). Rearing was carried out at 20 ±1°C, 75 ±5%RH and at a photoperiod of LD 18:6. Pupae were harvested, sexed and 10 pairs allocated (randomly selected) into 4 group oviposition units (at 15 ±1°C, LD 16:8). Eggs were collected fortnightly and incubated at 18 ±1°C and at LD 18:6. Eggs of 'blackhead' stage (250) were inoculated into 4 larval rearing units (Figure 2.1) and reared at 20°C until pupation. To study the effect of laboratory sub-colonisation, half of the neonate larvae during G_6 were randomly

inoculated into a diet composed of sheep nut (commercial sheep food supplement) and lima beans (SBD, see appendix Table A.5 for composition) and reared for seven generations under similar environmental conditions.

2.3.2 Life cycle studies on GPD and SBD diets

To identify changes occurring in the life cycle, 100 neonate larvae (see Figure 2.1) were randomly inoculated into polystyrene test tubes containing 1.5gm of GPD diet (and later neonates under sub-colonisation onto SBD diet). The rearing method was similar to that of Singh *et al.* (1985) for the Lightbrown apple moth, *Epiphyas postivittana*. The first twenty moths eclosing in each generation were individually paired (n=20 pairs) in plastic tubes (see appendix table A.2) and eggs laid were marked daily and later cut from the plastic oviposition surfaces for counting. Egg batches (n=20) were attached to the sides of translucent plastic Chinese containers by sticky labels and incubated at $18 \pm 1°C$, LD 18:6. The time for the first egg hatch was considered as the time of hatch for the rest of the batch. Eggs not hatched after 20 days were counted and percent hatch calculated for each generation. The mating status of females was ascertained by dissecting abdomens 10 days after pairing for the presence of spermatophore(s) in the bursa copulatrix. In cases where the spermatophore was present (indicating that mating had occurred) but where no fertile eggs had been laid, suspension of spermatophore content in physiological saline solution (0.75g

NaCl/100ml of distilled water) was made and checked under light microscope to confirm sperm motility. At the end of rearing of G_{11}, 25 wild larvae collected by sticky traps on Citrus and Kiwifruit branches in the Auckland area were reared to adults and their progeny (denoted G_W) reared in the laboratory for one generation to ascertain that similar changes had not occurred in the wild population.

2.3.3 Aspects of the life cycle monitored

Life cycle monitoring was carried out daily at about 0900hrs for duration (days) of complete larval, pupal and adult development (stadia duration, pupal periods and adult longevity for both male and female). Reproductive studies were carried out for fecundity, fertility (% of fertile eggs/female), number of egg batches /female, pre-oviposition period (days before the first egg batch is laid), oviposition period (days between first and last egg batch) as well % of mated females (females with spermatophore/20 females). Larval survival (% larvae pupating/total larvae) adult survival (% eclosing adults/total pupae), % egg hatch, hatching time, pupal weights and sex ratio were also calculated for each generation. No life cycle data were collected in G_7 and G_8; and $G_{7/2}$ and $G_{8/3}$ under sub-colonisation.

Indices used to monitor life cycle changes included pupation rate index (P_R= no of larvae pupated by the eclosion of the first

adult/total pupae). Pupal synchronism (S_P) and adult synchronism (S_A) indices were determined as the relative number coincidences of male and female larva pupating or adults eclosing simultaneously (J. H. Maindonald, pers. comm.).

The number of pupation coincidences is minimum ($\Sigma m_P, \Sigma f_P$), involving twice the minimum ($\Sigma m_P, \Sigma f_P$) larvae. This can be written as $\Sigma m_P + \Sigma f_P$ - ABS ($\Sigma m_P - \Sigma f_P$). The proportion of larvae pupating simultaneously is thus:-

$$S_P = \frac{(\Sigma m_P + \Sigma f_P - ABS\ (\Sigma m_P - \Sigma f_P))}{(\Sigma m_P + \Sigma f_P)}$$

= relative number of coincidences of male and female

larvae ($_P$) pupating simultaneously.

$$S_A = \frac{(\Sigma m_A + \Sigma f_A - ABS\ (\Sigma m_A - \Sigma f_A))}{(\Sigma m_A + \Sigma f_A)}$$

= relative coincidences of male and female adults

($_A$) eclosing simultaneously.

where Σm = sum of male frequency

Σf = sum of female frequency

ABS = Absolute value

S_P or S_A is 1 when the coincidences were equal, i.e. where each male pupating or eclosing had a female, and 0 when no coincidences

occurred. S_A is expected to be lower than S_P due to correction by shorter female pupal period. The synchronism indices are important because, though adult moths can be stored at 10-12°C, there is an approximately 50% reduction in mating results for each day of storage.

Analysis of life cycle data was done using basic descriptive statistics, dotplots, histograms, scatterplots and correlations to identify trends over successive generations. Analysis of of variance and t-tests using Minitab computer statistical data processing package (Ryan *et al.* 1985) were used to identify significant differences.

2.4 RESULTS

2.4.1 Colonisation on GPD diet

Table 2.1 is a summary of life cycle results on GPD. Analysis of the duration for each generation indicated no outstanding differences between the stadia of the five larval instars for both males and females. There were also no significant difference between G_1-G_{12} and G_W. Durations of L_2 and L_5 declined progressively (r= -0.52 and -0.74 respectively) over generations of rearing. Pupal period was significantly shorter (p< 0.001) for females compared to males in each generation. Pupal periods showed no significant trends though G_2 exhibited a significant rise (P< 0.001) over G_1

for both sexes. Total larval periods were longest in G_2 and tapered gradually to G_9. Adult longevity also exhibited no significant change in the 12 generations of rearing. Histograms of larval period indicated that only G_2 had slower rate of development (determined from the medians of the larval period). Figure 2.2 displays percent survival curves for larvae and pupae. Larval mortality occurred mostly within L_1 during larval establishment on diet and averaged 17.6 % for all the generations compared to 2% in G_W. Failure for adult eclosion (pupal mortality) exhibited no appreciable trend remaining at about 14% in each generation. Larval and pupal morphological malformations were rare averaging 0.9%, but more frequent for adults, gradually increasing from 5% in G_5 to 11% in G_{11}. Wing deformation was the common type of adult malformation. No morphological malformation was recorded in G_W. A red eyed mutation for both sexes was first noted at G_9 and averaged 8% in subsequent generations.

Male and female pupal weights did not trend over the generations. G_W had significantly higher female pupal weights ($p<0.01$) than G_1-G_{12}. The pupation rate index (P_R) averaged 1 indicating that all larvae had pupated by the eclosion of the first adult. Sex ratio at the pupal stage was skewed towards males in G_1, G_2-G_5, but towards females in G_2, G_6-G_{11}. Figure 2.3 displays dotplots of pupation and emergence patterns of G_{12} and G_W. Pupation was protandrous with males pupating earlier than females. Adult

synchronism index (S_A) was less than the pupal synchronism index (S_P) for all generations except in G_2 where it was equal. A comparison of synchronism indices of G_W with other generations showed no outstanding difference.

The pre-oviposition period averaged 7.7 ±1.5 (95% C.I.) days for mated females but were significantly longer ($p<0.001$) for non-mated females at 15.1 ±3.8 days. In all generations, the oviposition period for mated females was significantly longer ($p<0.01$) than for non-mated females. Pre- and oviposition periods showed no trends over successive generations. Fecundity was not significant and did not trend through successive generations (Figure 2.4). Number of egg batches averaged 2.1 ±2.1/female and did not vary in successive generations. Fertility decreased from G_5 to G_{12}. Spermatophore content indicated that the decrease in fertility was due to a decrease in sperm motility from 5% of the mated females with sperms showing no motility in G_5 to 50% in G_{11}. Mating propensity (% of females with multiple mating determined from spermatophore count) was low ranging from 3-6%. No more than two spermatophores were recorded in any mated female. There was an increase from 5% of mating adults failing to separate in G_9 to 20% in G_{12}. Failure for mating pairs to successfully separate occurred after the second or third mating attempt. Period to egg hatch remained relatively constant at 12.5 ±1.2 days while the percentage of egg hatch showed a decline from G_9 to G_{12}.

2.4.2 Sub-colonisation on SBD diet

The stadia of the five generations of sub-colonisation on SBD ($G_{6/1}$-$G_{12/7}$) were not significantly different from colonisation on GPD. Table 2.2 is a summary of data on sub-colonisation on SBD. The total larval period for each generation on SBD was longer for both sexes than for GPD. Longevity of SBD adults was generally enhanced for both sexes over GPD. The rate of development was slower than for GPD but had no trend in the 5 generations on SBD, though slowest in $G_{10/5}$ and fastest in $G_{11/6}$. Survival increased through the generations but tended to be lower than for GPD (see Figure 2.2). As with GPD, most larval mortality occurred within L_1 (averaging 17.2%). Pupal mortalities were lower than for GPD (5.4%), and morphological malformations absent. Pupal weights were only significant from GPD in $G_{11/6}$ ($p<0.05$) but not significant from G_W. P_R did not vary and sex synchronism was skewed towards males. The second generation of sub-colonisation was similar to G_2 on GPD as S_A was equal to S_P. Pre- and oviposition periods were not significant from GPD. Fecundity (Figure 2.4) was significantly ($p<0.01$) higher than for GPD only in $G_{6/1}$. Number of egg batches/female averaged 10.8 ± 3.7/female. All mating pairs succeeded in separation. The sub-colony showed an increase in multiple mating (in $G_{12/7}$, 60% of the mated females had multiple mating, 36% of the multiple mated had three spermatophores each.

No red eyed individuals developed under sub-colonisation on SBD. To test whether the development of the red eye in the GPD colony was a true mutation and not due to dietary effects, male and female red eyeds; normal males and female red eyeds as well as red eyed males and normal females were crossed (n=10 pairs) for a generation and reared on SBD diet. In each case over half the progeny was red eyed, with no preference for sex. It is highly likely therefore that these were true mutants.

To confirm that decrease in sperm motility was linked to males of the GPD colony, and that GPD females were normal, GPD males were crossed with SBD females; and GPD females with SBD males (n=10 in each case). Fecundity was 73.6 ±28.9 eggs/female, 37.5 eggs/female were fertile and a 100% mating for the GPD males X SBD females. There was one multiple mating (with 3 spermatophores) and 70% of the females had spermatophores with sperms that were not motile. The SBD males X GPD females exhibited significantly higher fecundity (p<0.01 for 289.3 ±27.7; 230.7 eggs/female were fertile) and with three multiple mating (with 2 spermatophores each).

2.5 DISCUSSION

2.5.1 Classical definition of adaptation

An adaptation has been defined as an advantageous

characteristic to an individual organism or to the conspecific group among which it lives. Adaptation, or the process of adaptation is the acquisition within a population of such genetically fixed advantages (Dobzhansky, 1941, 1970; Simpson, 1953; Lewontin, 1974; Edwards, 1977, Lewontin, 1978; Berry, 1982). Adaptation has an interaction at four levels; the physical environment, the extrademe biotic environment, the deme, and the individual (Simpson, 1953).

True adaptation (Darwinian) is a product of genetic change, producing 'new' individuals. Adaptation in this sense should not be allowed to occur in insect rearing systems, where the identity of colonised insects with the wild population must be maintained. Such adaptations ('misadaptations' according to Rössler, 1975) would hinder biological control programmes as laboratory reared insects would have to re-adapt to their former habitats (Bush, 1979; Curtis, 1985) or be reproductively isolated (Mayr, 1963; Boller, 1972, 1979). Any results obtained from use of such adapted insects for research would be less likely representative of the original population. Gould and Lewontin (1979) identified two other phenomena commonly referred to as adaptation; phenotypic plasticity (non heritable, but permitting organisms to mould to prevailing circumstances during ontogeny) and cultural adaptation (heritability imposed by learning in social species).

In the colonisation of *C. jactatana*, no changes were

observed in the life cycle that were true adaptations. Changes in %
mating, sperm motility, fertility, failure of adults to separate, egg
hatch and abnormal morphs can be explained as dietary
incompatibility. Similar changes in the life cycle were absent under
sub-colonisation on SBD. Red eye is probably a mutation due to GPD
diet and not a true adaptation (akin to the white-eyed flies noted
by Berlocher and Friedman (1981), which had lower heterozygosity
than normal laboratory flies). The major difference in composition
of SBD from GPD is in the brewers yeast it contains (though found
feeding on similar fruit trees in the wild as the other tortricid
leafrollers e.g. Lightbrown apple moth, *C. jactatana* has the habit of
feeding on dead, decaying or necrotic leaf tissue (J. S. Dugdale
pers. comm.). These findings are collaborated by Tignor and Eaton
(1986) who found minimal effects of prolonged colonisation on
cabbage loopers. None of the effects were true adaptations.

2.5.2 Ranges of colonisation

Life processes are strongly limited by abiotic conditions
(e.g. temperature, photoperiodism, humidity, wind velocity) and
hence organisms perform efficiently under a narrow band of magnitude
of environmental variables (Putman, 1984; Putman and Wratten,
1984). Shelford (1913) described bell-shaped performance curves
(tolerance curves) with peaks which represent optimal performance
and with tails indicating limits of performance where death occurred
for any physiological or chemical process (lethal limits). Putman,

(1984) and Putman and Wratten (1984) defined a series of inner performance limits (nesting limits) which included critical maxima and minima where organisms though not dead, are inviable. Within critical limits are preferred ranges surrounding the optimum (see Figure 2.5). These curves are, however, not fixed and organisms will adjust over time in order to bring their optimum level of performance in tune with the equilibrium of the operating ranges. This process has been described as acclimatisation, a process of change in enzyme systems according to Putman (1984) and Hoffmann (1985). Acclimatisation can occur within hours, within days or over successive generations (periods that are all together very much shorter than evolutionary time scale).

Organisms may tolerate a range of environmental factors due to reserves of inherent variability in their genetic make up whose fundamental origin is in mutation (Berry, 1982). Genetic variation usually maintained by a balance of selective forces, may be irretrievably lost (decay) in small populations due to random events such as genetic drift (Berry, 1982; Joslyn, 1984; Bartlett, 1984b). Loss of variability in insects has been demonstrated to be harmful (Boller, 1972; 1979; Bush, 1975; Mason *et al.* 1987). Berlocher and Friedman (1981) measured the genetic variation of *Phormia regina* and found laboratory flies possessed less variation than wild flies. Conservation of genetic variation should therefore be a prime strategy for laboratory colonisation and subsequent

rearing. Van Lenteren (1986) proposed 'precolonisation' as a measure (selection and pooling of founder insects from a wide range) to increase the variability of laboratory insects.

I propose that for colonisation to be successful conditions need to fall within the tolerance limits of the insect. Two ranges are proposed for colonisation (Figure 2.5). The larger range is the limit of colonisation within which is an ideal or preferred range. The ranges of colonisation are dependent on the habits of different insect species and reflect their level of phenotypic or somatic plasticity (see Lewontin, 1965; Gould and Lewontin, 1979). The ranges will be narrower in specialist insects (e.g. parasitoids) and consequently such insects are difficult to colonise in the laboratory as they can only exploit a narrow range of colonisation (less plastic). Generalist insects have a wider (more plastic) range of colonisation and consequently are more readily colonised. Haefner's experiments (1970) on the interactive effects of different variables in deriving tolerance curves indicates that the ranges of colonisation may shift depending on levels of interaction.

Understanding ranges of colonisation allows for 'capture' of the inherent variability. Fluctuating temperatures, photoperiods with dawn and dusk effect are superior to constant temperature or sudden photoperiods as they enhance more 'capture' of a population's

variability (Bush and Neck, 1975; Economopoulos and Loukas, 1986). Such fluctuations represent wider ranges of colonisation than constant conditions. Decay of variability may be delayed by use of random mating systems so that the probability of getting a cross between the genotype depends wholly on their relative frequencies (Edwards, 1977; Berry, 1982; Stock and Roberts, 1982; Bartlett, 1985). The success and ease in colonisation *C. jactatana* was due to choice of variables that were within the insect population's preferred range of colonisation. Choice of rearing temperature (18 and 20°C) and photoperiod (LD 18:6) was realistic because of successful use with two leafrollers, *Epiphyas postivittana* (Walker) and *Planotortrix excessana* (Walker) (Singh *et al.* 1985; Hobson and Singh, 1987).

2.5.3 Adaptation or selection, acclimatisation and domestication ?

The problem with the common interpretation of the 'production curve' (Boller, 1972) is that it is indicative of a process of true adaptation during the recovery phase. As no changes in the colonisation of *C. jactatana* can be described as adaptive in the Darwinian sense, a possible explanation for the 'production curve' is one of selection, acclimatisation and domestication. The process of selection has been compared to a sieve (Dobzhansky, 1970; Bartlett, 1984b) where nothing new is created, only loss of ill adapted variants and retention of those better adapted to the selecting environment. Selection and adaptation are not in any way analogous.

Early in the colonisation phase a process of selection ('winnowing' according to Bartlett 1984) occurs where individuals with genotypes that favour a rigorous exploitation of the new habitat (and hence more fit, Edwards 1977), survive and reproduce (Dobzhansky 1941 described this sector of the population as 'genetically effective'). Though initially some individuals are better adapted to the conditions, their selection during colonisation and subsequent spread is not adaptation. This is in agreement with Pashley and Proverbs (1981) who identified selection as a possible cause for gradual changes in allozyme frequency.

It is my thesis that the 'production curve' is an indicator of the width of the ranges of colonisation. The magnitude of decline and recovery in performance of aspects of the life cycle indicates the width of ranges of the conditions offered during colonisation. Where the range is narrow, the decline is steep, the recovery is gradual and delayed. Where the range is wide, the decline is slight, and recovery is rapid and immediate. Nei *et al.* (1975) calculations showed that ranges of colonisation ('size of bottleneck') are not the only factors in the reduction of variability. The rate of population growth after colonisation has profound effect on the conservation of variability. The faster the rate of growth, the less the loss of heterozygosity.

The 'production curve' is only applicable to the results of

larval and pupal survival in the colonisation and sub-colonisation of *C. jactatana* (Figure 2.2) which show slight decline and rapid recovery phases. In each case, recovery was within a generation indicating that colonisation was accomplished with ease. The rapid increase in the laboratory population within the first three generations might have aided conservation of variability. Sub-colonisation was less traumatic producing no definite decline in larval and pupal survival.

During laboratory colonisation of insect species three processes are proposed to occur; selection, acclimatisation and domestication. Individuals not within ranges of colonisation, but within the lethal limits will die (the first 'bottleneck', see Boller 1979). Individuals within critical limits may survive but will not breed successfully. Only individuals within the preferred range of colonisation will breed in the first generation. Selection therefore occurs for those individuals that perform best (Mackauer, 1980). Individuals with optimal performance at the edge of the preferred range of colonisation, may be able to breed but show low levels of performance. Such individuals may have longer developmental periods, low pupal weights or low reproductive ability. In successive generations a process of acclimatisation (due to phenotypic plasticity of the species) gradually shifts the optimal performance to within the preferred range of colonisation. The rate of acclimatisation is not uniform for all individuals, some

acclimatise faster than others hence there is a gradual improvement in biological performance in subsequent generations. The process of selection therefore compliments acclimatisation.

Leppla *et al.* (1983) showed that three levels of colonisation of the Mediterranean fruit fly (rearing with care; easing into culture by successive removal of normal stimuli and forcing directly into production) had varying effects on the pupal weight. The three levels represent three different ranges of colonisation offered. Changes in diel periodicity that Leppla *et al.* (1976) noted are acclimatisation and not adaptation as reported. Koyama *et al.* (1986) experiments showing that high light intensity of the laboratory caused artificially reared melon flies to initiate mating earlier although copulation and courtship were not affected (no sexual isolation) are also examples of acclimatisation and not adaptation. Earlier oviposition of the tobacco budworm, *Heliothis virescens* (Raulston, 1975; Roush, 1986) is an example of selection complimenting acclimatisation

The third process in colonisation is domestication. Domestication is non-genetic (though interacting with gene make up) referring to the gradual 'acceptance' of insect species to conditions in the new habitat. Such conditions have no direct involvement with the physiological or biochemical responses of the various life processes. Domestication is 'getting accustomed' to human odour,

human presence, sounds from refrigerators, incubators, airflows, telephones, smell of chemicals (e.g. alcohol, formalin or sodium hypochlorite used for disinfection) and containers and substrates (for larval rearing and adult oviposition). Domestication is the 'acceptance' by insect species of the new distractions and being able to perform in spite of them. Domestication is synonymous with conditioning (change of behaviour due to environmental factors that does not change the genotype; Boller, 1972). Freshly collected larvae and adults of wild *C. jactatana* tried to escape through cotton plugs in rearing of G_W. Such attempts gradually became infrequent and stopped within 10-20 days and were not noticed in subsequent generations. As no larvae or adult escaped, the decline in escape attempts is not due to selection, but a gradual 'acceptance' of the rearing containers as part of the new habitat.

2.6 CONCLUSION

Monitoring the life cycle of *C. jactatana* in successive generations showed no changes that were adaptive in the Darwinian sense. Laboratory colonisation occurs through processes of selection, acclimatisation and domestication. The 'production curve' is interpreted as an indicator of the width of ranges of colonisation. An proposed optimal strategy to ascertain conservation of laboratory insect's genetic variability is the use of environmental factors with wide ranges during colonisation.

Table 2.1: Life cycle over successive generations

of laboratory rearing of *C. jactatana* on

GPD diet

* at 18 ±1°C, 75 ±5%RH, Adult longevity

at 15 ±1°C, 75 ±5%RH

** mean «±» 95% confidence intervals

Rows followed by the same letter are not

significantly different (p>0.01)

		G_1	G_2	G_3	G_4	G_5	G_6	G_8	G_{10}	G_{11}	G_{12}	Wild
N	♂	29	49	51	43	29	36	41	41	30	50	54
	♀	18	48	43	35	34	40	48	49	52	44	44
Duration of L_1 (days)	♂	6.5a** 6.3<>6.7	-	6.4a 5.6<>7.2	-	-	7.8a 6.8<>8.8	6.7a 5.9<>7.5	9.4a 8.5<>10.3	7.8a 6.9<>8.7	6.5a 6.1<>6.9	7.2a 6.9<>7.5
	♀	6.8a 6.3<>7.3	-	5.9a 5.7<>6.1	-	-	7.3a 6.3<>8.3	6.6a 6.1<>7.1	8.5a 7.9<>9.1	7.7a 6.9<>8.5	6.7a 6.4<>7.0	7.9a 7.5<>8.3
Duration of L_2 (days)	♂	5.0a 4.6<>5.4	-	7.7b 7.0<>8.4	-	-	5.5a 5.1<>5.9	5.3a 5.0<>5.6	5.1a 4.5<>5.7	5.7a 5.3<>6.1	4.6a 4.4<>4.8	4.8a 4.6<>5.0
	♀	5.4a 4.7<>6.1	-	8.4b 7.4<>9.4	-	-	5.8a 5.2<>6.4	5.5a 5.3<>5.7	4.9a 4.5<>5.3	5.7a 5.4<>6.0	4.5a 4.3<>4.7	4.8a 4.5<>5.1
Duration of L_3 (days)	♂	4.0a 3.8<>4.2	-	5.6b 5.2<>6.0	-	-	4.6a 4.3<>4.9	3.8a 3.3<>4.3	4.6a 4.3<>4.9	4.0a 3.6<>4.4	4.5a 4.3<>4.7	4.7a 4.5<>4.9
	♀	4.4a 3.8<>5.0	-	6.0b 5.3<>6.7	-	-	4.6a 4.4<>4.8	3.4a 3.0<>3.8	4.8a 4.6<>5.0	4.2a 3.9<>4.5	4.8a 4.5<>5.1	4.8a 4.6<>5.0
Duration of L_4 (days)	♂	4.7a 4.4<>5.0	-	5.4a 5.2<>5.6	-	-	4.8a 4.5<>5.1	4.1a 3.8<>4.4	4.9a 4.6<>5.2	4.6a 4.3<>4.9	4.9a 4.8<>5.0	5.3a 5.0<>5.6
	♀	4.6a 4.2<>5.0	-	6.1b 5.6<>6.6	-	-	5.2a 5.0<>5.4	4.5a 4.2<>4.8	5.1a 4.8<>5.4	4.9a 4.7<>5.1	5.2a 5.0<>5.4	5.9b 5.6<>6.2
Duration of L_5 (days)	♂	11.4a 10.9<>11.9	-	14.5b 13.6<>15.4	-	-	11.5a 10.8<>12.2	9.7a 7.7<>11.7	12.0a 11.2<>12.8	10.8a 9.7<>11.9	12.4a 11.7<>13.1	12.9a 12.3<>13.5
	♀	12.6a 12.0<>13.2	-	16.0a 14.7<>17.3	-	-	13.4a 12.6<>14.2	11.5a 10.7<>12.3	13.2a 12.3<>14.1	11.6a 10.8<>12.4	13.2a 11.7<>14.7	13.7a 13.1<>14.3
Mean Larval period (days)	♂	31.5a 30.8<>32.2	43.8b 42.2<>45.4	40.7b 39.4<>42.0	36.7c 35.5<>37.9	36.1c 34.8<>36.8	35.2c 34.2<>36.2	31.7a 30.7<>32.7	37.3c 35.9<>38.7	33.8c 32.9<>34.7	33.9c 33.4<>34.4	35.9c 35.4<>36.4
	♀	34.1a 32.9<>35.3	44.6b 43.0<>46.2	43.3b 41.9<>44.7	37.1c 35.8<>38.4	37.8c 36.6<>39.0	37.1c 36.1<>38.1	32.5a 31.9<>33.1	37.5c 36.8<>38.2	35.1a 34.4<>35.8	35.4a 34.6<>36.2	37.8c 37.3<>38.3
Mean pupal weights (mg)	♂	30.6a 29.4<>31.8	31.0a 29.3<>32.7	31.8a 30.6<>33.0	30.7a 28.9<>32.5	32.0a 29.9<>34.1	33.7a 30.9<>36.5	31.2a 30.0<>32.4	29.5a 28.2<>30.8	34.3a 32.8<>35.8	30.3a 29.3<>31.3	36.0a 34.7<>37.3
	♀	39.9a 37.9<>41.9	41.7a 40.1<>43.3	43.1a 41.5<>44.7	41.8a 39.2<>44.4	43.4a 41.6<>45.2	45.0a 43.6<>46.4	44.6a 43.6<>45.6	42.3a 41.0<>43.6	46.5a 45.0<>48.0	41.3a 39.7<>42.9	50.4b 48.1<>52.7
Pupal period (days)	♂	15.5a 15.2<>15.8	20.8b 19.8<>21.8	19.1b 18.9<>19.3	17.5b 17.0<>18.0	18.1b 17.8<>18.4	18.1b 17.8<>18.4	15.3a 15.0<>15.6	18.6b 18.3<>18.9	15.8a 15.5<>16.1	18.6b 17.5<>19.7	16.8b 16.4<>17.2
	♀	14.6a 14.0<>15.2	19.8b 19.1<>20.5	18.1a 17.6<>18.6	16.6a 16.2<>17.0	16.7a 16.1<>17.3	16.6a 16.4<>16.8	15.0a 14.4<>15.6	16.8a 16.4<>17.2	14.7a 14.1<>15.3	16.6a 15.0<>18.2	15.5a 14.8<>16.2
Adult longevity (days)	♂	20.4a 16.7<>24.1	16.8a 15.3<>18.3	16.3a 15.0<>17.6	14.7a 13.8<>15.6	16.3a 12.6<>20.0	21.9a 18.5<>25.3	22.7a 20.6<>24.8	17.1a 15.9<>18.3	21.0a 18.7<>23.3	25.7a 24.1<>27.3	30.1b 28.4<>31.8
	♀	22.7a 19.7<>25.7	21.5a 19.2<>23.8	22.4a 20.8<>24.0	20.6a 18.4<>22.8	19.0a 16.8<>21.2	24.1a 19.0<>29.2	29.8a 25.9<>33.7	24.3a 21.9<>26.7	25.3a 22.7<>27.9	28.7a 26.9<>30.5	28.2a 27.0<>29.4
Sex ratio ♂:♀		1:0.7	1:1.2	1:0.8	1:0.8	1:0.9	1:1.2	1:1.7	1:1.7	1:1.8	1:0.9	1:0.8
Percentage of mated females		50	47	69	53	50	80	40	50	60	95	31
Percentage of egg hatch		74	-	-	-	-	-	77	67	68	55	82
Pupation rate index (P_m)		1.0	0.96	0.97	0.99	0.98	1.0	1.0	0.98	1.0	1.0	1.0
Pupal Synchronism index (S_p)		0.26	0.72	0.48	0.54	0.54	0.50	0.40	0.59	0.56	0.64	0.57
Adult Synchronism index (S_A)		0.57	0.72	0.51	0.78	0.62	0.66	0.72	0.78	0.66	0.70	0.73

Table 2.2: Life cycle over successive generations

of sub-colonisation of *C. jactatana* on

SBD diet

* at 18 ±1°C, 75 ±5%RH, Adult longevity

at 15 ±1°C, 75 ±5%RH

** mean «±» 95% confidence intervals

Rows followed by the same letter are not

significantly different (p>0.01)

		Generations in laboratory/sub-colonisation*				
		'G$_{6/1}$'	'G$_{9/4}$'	'G$_{10/5}$'	'G$_{11/6}$'	'G$_{12/7}$'
N	♂	36	37	41	39	35
	♀	39	43	40	52	50
Mean Larval period (days)	♂	37.7a** 36.5«±»38.9	37.5a 35.4«±»39.6	43.2b 41.9«±»44.5	33.9c 33.0«±»34.8	38.9a 37.8«±»40.0
	♀	40.8a 39.6«±»42.0	40.3a 37.8«±»43.0	44.4a 42.9«±»45.9	35.1b 34.4«±»35.8	40.5a 39.3«±»41.7
Mean pupal weights (mg)	♂	31.1a 29.9«±»32.3	29.7a 28.7«±»30.7	30.9a 30.0«±»31.8	38.1b 36.4«±»39.8	30.2a 29.4«±»31.0
	♀	41.8a 40.2«±»43.4	38.7b 37.7«±»39.7	43.2a 42.0«±»44.4	50.5c 49.2«±»51.8	42.1a 41.3«±»42.9
Pupal period (days)	♂	18.2a 17.9«±»18.5	18.0a 17.5«±»18.5	16.4b 15.9«±»17.1	15.2c 15.0«±»15.4	18.5a 18.2«±»18.8
	♀	16.4a 16.0«±»16.8	17.3a 16.6«±»18.0	15.4a 15.0«±»15.8	15.8a 15.6«±»16.0	16.7a 16.5«±»16.9
Adult longevity (days)	♂	26.0a 24.2«±»27.8	28.3a 23.6«±»33.0	27.5a 25.4«±»29.6	21.0b 18.8«±»23.2	30.3a 28.7«±»31.9
	♀	26.6a 25.4«±»27.8	30.6a 26.3«±»34.9	26.8a 24.2«±»29.4	19.9b 17.2«±»22.6	29.8a 28.3«±»31.3
Sex ratio ♂ : ♀		1:0.9	1:1.6	1:0.9	1:1.4	1:1.7
Percentage of females mated		100	80	65	89	75
Percentage of egg hatch		93	–	–	77	81
Pupation rate index (P$_R$)		1.0	0.88	1.00	1.00	1.00
Pupal Synchronism index (S$_P$)		0.26	0.72	0.48	0.54	0.54
Adult Synchronism index (S$_A$)		0.57	0.72	0.51	0.78	0.62

Figure 2.1: Random mating system used for rearing *C. jactatana* 12 successive generations

Larval rearing units

| 1 | 2 | 3 | 4 |

Pupae
sexed

+ 10 ♂ 10 ♀ + 10 ♂ 10 ♀ + 10 ♂ 10 ♀ + 10 ♂ 10 ♀ +

Group oviposition units

| 1 | 2 | 3 | 4 |

Eggs

Egg incubation units

| 1 | 2 | 3 | 4 |

Neonate
larvae

25   +   25   +   25   +   25 = 100
                                          »»»»
                                      for lifecycle study

+ ½   ½ + ½   ½ + ½   ½ + ½   ½ +

Inoculated into larval rearing units
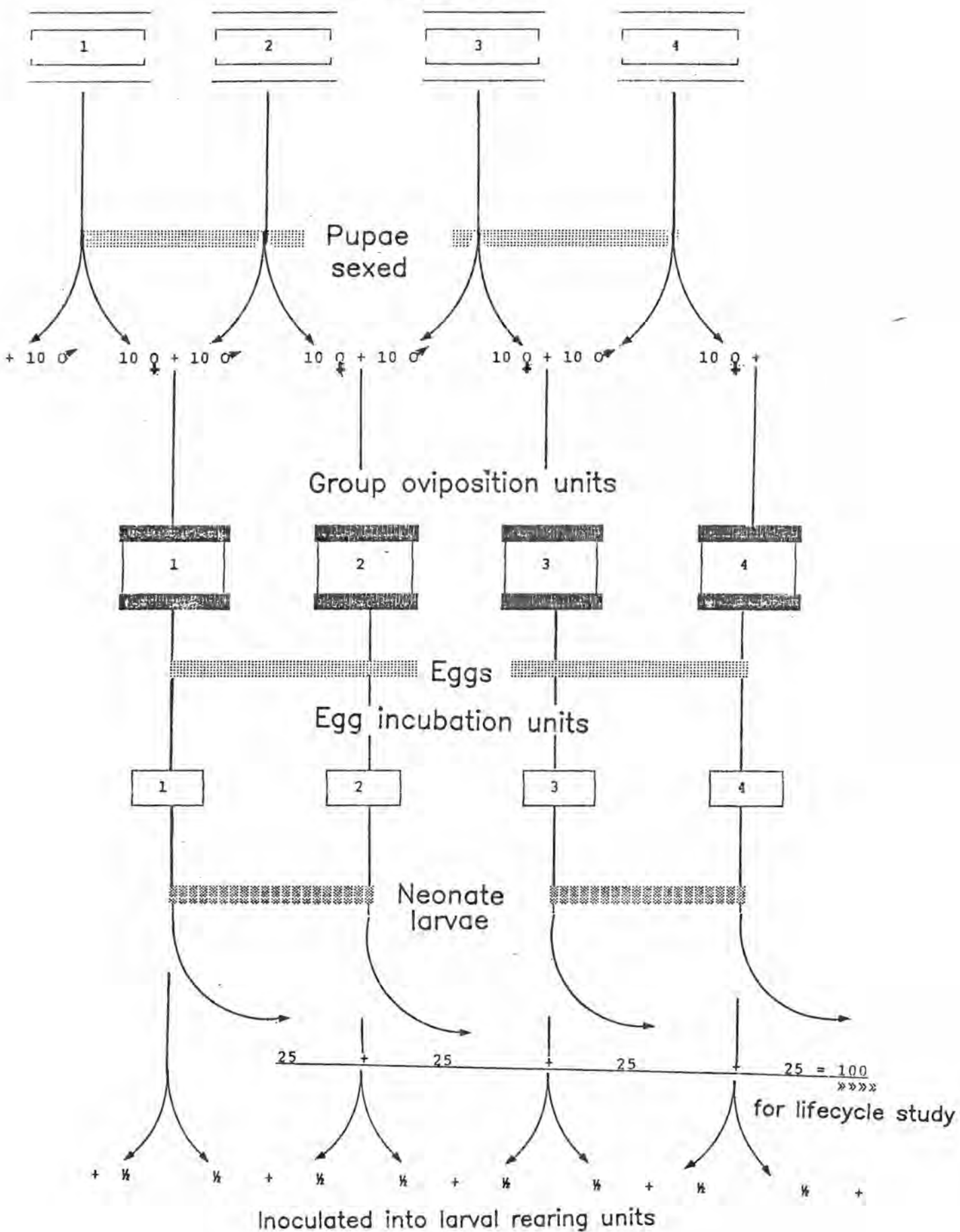
Figure 2.2: Larval and pupal survival over 12

successive generations

(see Tables 2.1 and 2.2 for sample size)
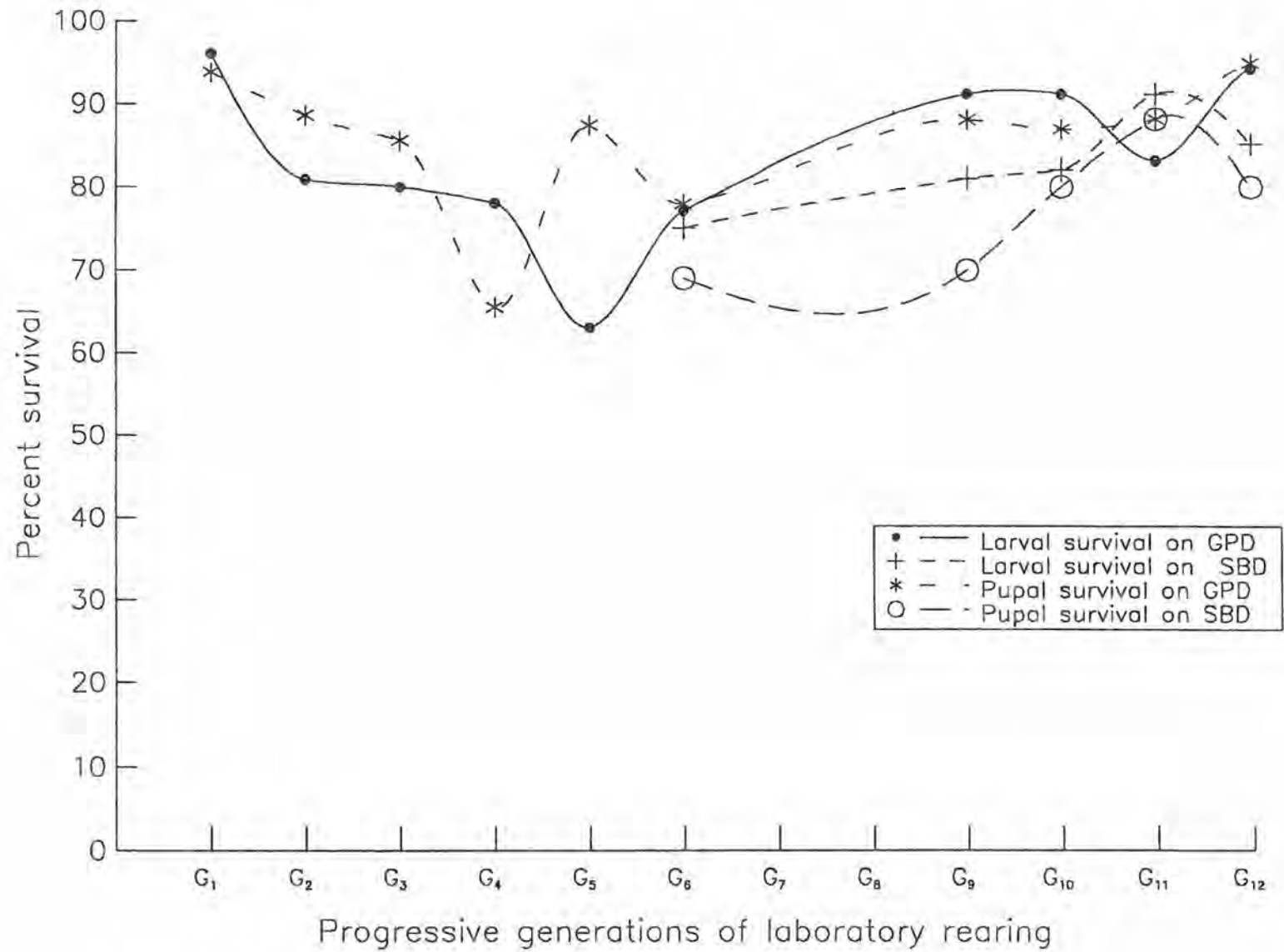
Figure 2.3: Sex synchronism in generation 12 and wild

C.jactatana

(see Tables 2.1 and 2.2 for sample size)

Generation 12

Wild

Pupation $S_P = 0.57$

Frequency (No)

Pupation $S_P = 0.64$

Eclosion

Eclosion $S_A = 0.70$ $S_A = 0.73$

Days from inoculation of neonates

35   40   45   50   55   35   40   45   50   55
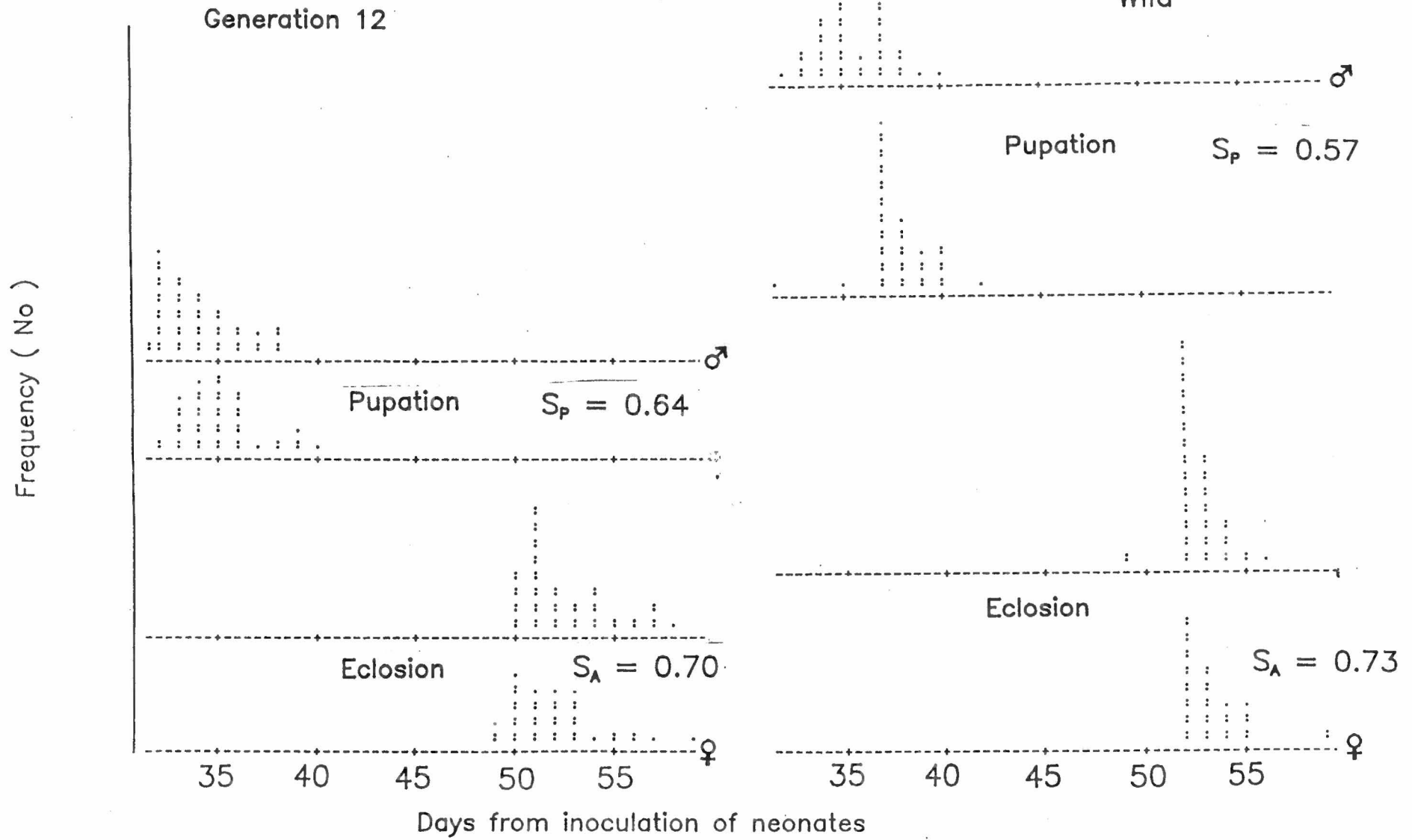
Figure 2.4: Fecundity of *C.jactatana* over 12

successive generations

Vertical bars represent 95% confidence intervals

(Based on 20 females in each generation)
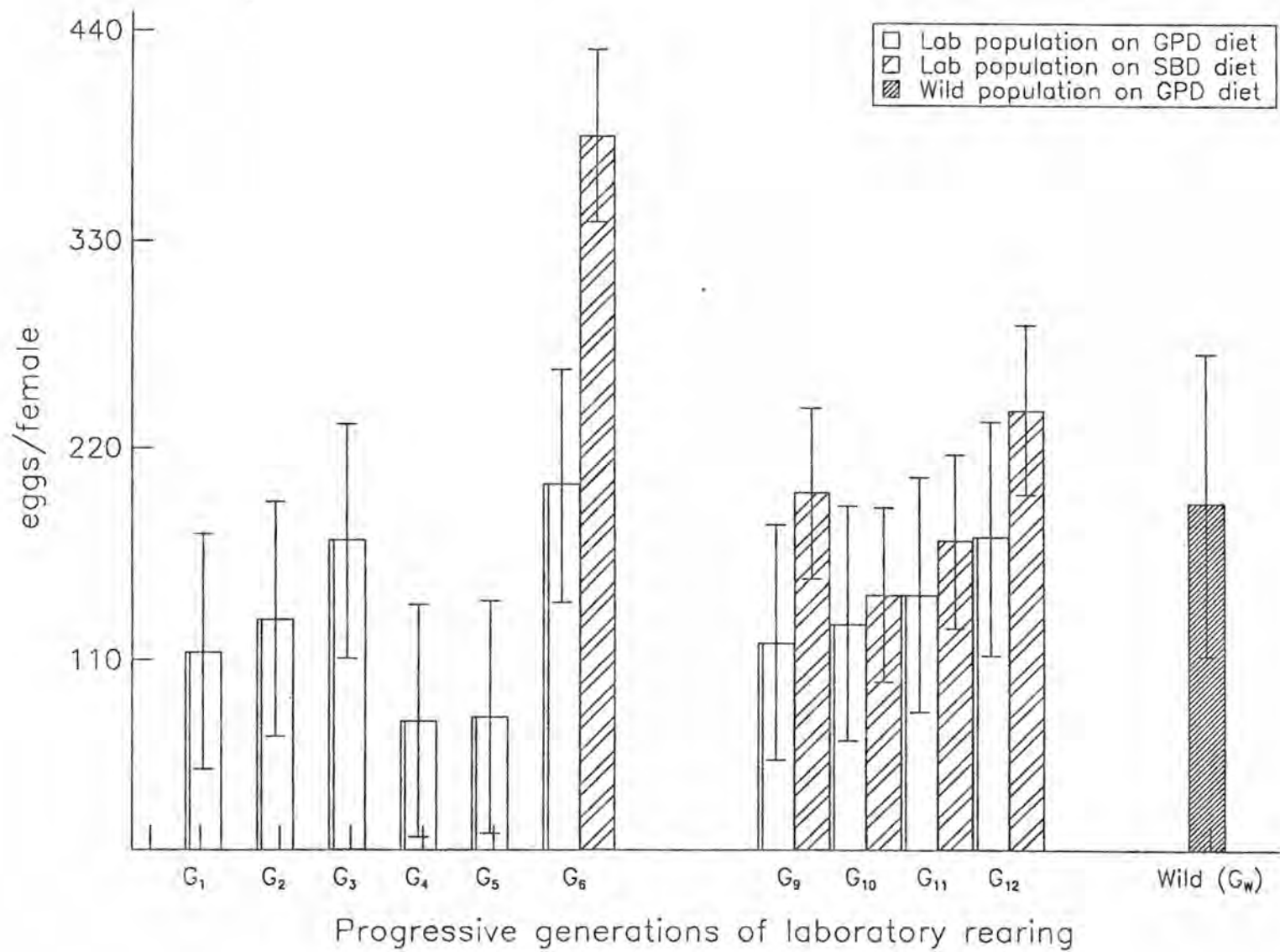
Figure 2.5: Ranges for colonisation

(Modified after Putman (1984) and Putman and
Wratten (1984)
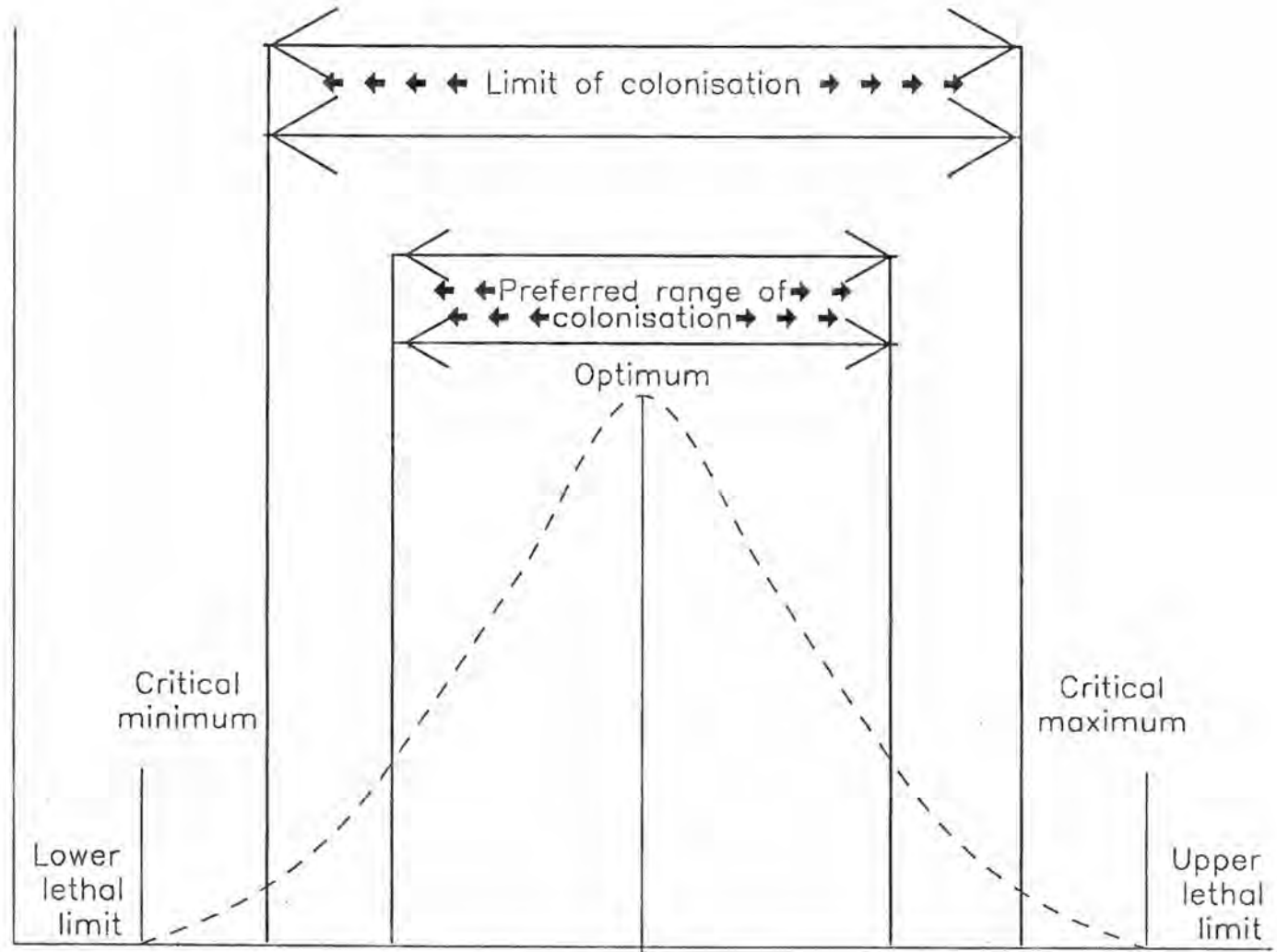
CHAPTER / 3

CRITICAL, PUPAL AND ADULT WEIGHTS IN THE SIZE RELATED
METAMORPHOSIS OF THE BLACK LYRE LEAFROLLER 'Cnephasia'
jactatana (Walker) (LEPIDOPTERA: TORTRICIDAE)

3.1 ABSTRACT

Adult reproductive performance is linked to the period of
feeding done by the final instar larva after attainment of a larval
critical weight (L_{CW}). The highest weight attained by a final
instar larva is referred to as the larval maximum weight (L_{MW}) and
is the onset of the pre-pupal period. The relationships between L_{CW},
pupal weight (P_W) and adult weight (A_W) are described as
functions of the L_{MW}. In the leafroller 'Cnephasia' jactatana, L_{CW}
was about 29mg and 36mg for male and female larvae of 1.18mm and
1.20mm head capsule width respectively. L_{CW} was dependent on larval
size and was approximately 75% of the mean L_{MW}. Over successive
generations of laboratory rearing, P_W was about 30% and 25% lower
than the L_{MW} for males and females respectively. A_W was consistently
about 50% and 40% lower than the P_W for males and females
respectively. The decrease in weight from L_{MW} to P_W was named as the
constant D_P and found to be 0.3 for males and 0.25 for females. The
total decrease from L_{MW} to A_W was the constant D_A and was 0.6 for
males and 0.5 for females. The duration of the latent feeding period

was positively correlated to P_W and A_W. Assuming that L_{CW} describes the L_{CW} for the smallest member of a laboratory population, quality indices can be derived that describe and predict pupal and adult performance.

3.2 INTRODUCTION

The final larval moult in holometabolous insects is a cataclysmic event involving an interaction of at least three hormones, photocycles, temperature, nutrition, body size and instar duration (Nijhout, 1979, 1981; Palmer, 1982; Safranek, 1984a). The timing of the final moult is crucial as it ends an active feeding phase and therefore has direct influence on adult size and biological performance (pupae and adults of Lepidoptera do not increase in size; feeding in adults is restricted to water intake for sustenance, although Gunn and Gatehouse (1985) showed that water uptake was necessary in egg hydration of some insect species).

The programme of events leading to pupation in holometabolous insects can be summarised as in Figure 3.1. Larvae probably identify their final instar by monitoring their size through some form of allometry until a threshold size is reached (Nijhout, 1975; Safranek and Williams, 1984b). Final instar larvae feed from the day of moult until they attain a larval critical weight or size (hereafter referred to as L_{CW}; see Nijhout and Williams, 1974a; Safranek and

Williams, 1984a). The critical size in itself is not causative of moulting but triggers the release of an inhibiting substance from an unknown area in the abdomen that prevents the brain secreting corpora allata-stimulating factor (allatropin) but which initiates the the secretion of corpora allata-inhibiting factor (allatohibin; Bhaskaran *et al.* 1980; Williams, 1975; based on work on the tobacco hornworm, *Manduca sexta*). Allatohibin acts on the corpora allata to stop any further secretion of juvenile hormone. It has been shown that the half life or decay of the juvenile hormone takes about $1\frac{1}{2}$ days in *M. sexta.* During this 'latent' or 'lag' period (Nijhout and Williams, 1974b) the larvae continue feeding, attaining weights above the L_{CW}. After decay of the juvenile hormone, the brain is able to secrete prothoracicotropic hormone (PTTH). Release of PTTH occurs in two surges. The first release induces the prothoracic gland to start secreting ecdysone, which is then converted into the moulting hormone 20-hydroxyecdysone (ecdysterone). It is the presence of the moulting hormone that causes larvae to stop feeding and to begin wandering, searching for pupation sites (Truman and Riddiford, 1974; Nijhout and Williams, 1974a; Jones *et al.* 1981). The second release of PTTH occurs later (approximately 2 days for *M. sexta,* Truman and Riddiford, 1974; and one day for the cabbage looper, *Trichoplusia ni,* Jones *et al.* 1981) and triggers another ecdysteroid peak leading to cuticle synthesis and ocellar pigment retraction, culminating in ecdysis (See Wigglesworth, 1970; Williams, 1975; Riddiford, 1980; Bhaskaran, 1981; Truman and Taghert,

1981; Nijhout, 1981; and Bollenbacher, 1985 for reviews). Gilbert *et al.* (1980) considers that it is the juvenile hormone that determines the onset and nature of the moult. High concentrations lead to a larval-larval moult and lower titres lead to a larval-pupal metamorphosis. Pupal-adult metamorphosis occurs in the absence of juvenile hormone.

Slansky and Scriber (1985) considered that the latent period between achieving L_{CW} and the actual cessation of feeding permits the larva to reach an 'ideal' or optimum weight if food quality, food quantity and environmental factors are suitable. L_{CW} is therefore only a minimal weight for pupation that allows production of a 'functional' adult (competent to survive and reproduce). The size or weight achieved by a larva above L_{CW} is the major factor that dictates the adult reproductive performance and hence, overall quality (ability to survive and colonise) of the insect.

Pupal weight (P_W) is commonly monitored in laboratory insect populations and is regularly incorporated into process and product analysis of production systems as a measure of quality (Chambers and Ashley, 1984). Using the native New Zealand leafroller, *Cnephasia jactatana* (Walker), this study set out to:-

1) test for the existence of a L_{CW} in the final instar as a threshold for pupation.

2) To establish relationships between L_{CW}, pupal and adult weight, latent feeding period and reproductive performance.

3.3 MATERIALS AND METHODS

Fifth instar (L_5) larvae were obtained from a laboratory population reared on GPD artificial diet (Singh, 1983; see appendix Table A.4 for composition and chapter 2 for rearing methods and management) for ten to twelve generations (G_{10}-G_{12}). As a preliminary study, medium sized larvae (1.18-1.20mm head capsule width) moulting to L_5 (day 0) were weighed and starved for 20 days in plastic vials (40 X 10mm). Larvae which had not pupated after 20 days were transferred onto artificial diet until pupation whereupon their sex was determined.

Other L_5 larvae (n=30 in each case) were allowed to feed for 1, 2, 3, and 4 days before starvation. All larvae starved after day 0 failed to pupate while only 50% of those starved after day 1 pupated. All larvae starved after two days of feeding pupated successfully. To precisely establish L_{CW}, 100 medium sized larvae of various weights which had fed for 12-24hrs after moult were starved for 20 days in G_{10}-G_{12}. In each case, those larvae failing to pupate after 20 days were transferred onto artificial diet until pupation. To evaluate the effect of the latent feeding period on adult

reproductive performance, pupae formed from all starved larvae were weighed and incubated until eclosion (where sex was again verified), females weighed and mated in perspex tubes at $15 \pm 1°C$, $75 \pm 5\%RH$ (see appendix Table A.2) with average sized males (of about 30mg pupal weight) derived from the stock population. The number of eggs laid were counted and longevity was determined for each female.

To determine weight gain over consecutive days in L_5, 90 larvae (freshly moulted into L_5 between 0080-1800hrs of day 0) were measured for head capsule width and transferred into plastic vials containing 1.5g of artificial diet. Each larva was thereafter daily weighed individually between 1200-1300hrs. A close observation was kept for morphological markers associated with changes in wet weight. Similar measurements were made for G_{11} and G_{12}. Progeny of wild collected larvae (G_W) were similarly measured to test for differences with the laboratory population.

Measurements of head capsule were taken of the greatest width (occipital foramen region) using a calibrated eyepiece micrometer mounted on a dissecting microscope. Individual weighing of L_5, pupae and adults was done using a Shimadzu electronic balance AEL-160 with an accuracy of $\pm 0.1mg$. Rearing and experimentation was conducted at $20 \pm 1°C$, $75 \pm 5\%RH$ with a photoperiod of LD 18:6.

3.4 RESULTS

Larvae *C. jactatana* undergo 4 moults in about 21 days at 20 °C. Growth is relatively uniform with head capsule width increasing from 0.23mm at L_1 to 1.19 ± 0.02mm for males and 1.24 ± 0.01mm for females at L_5. Head-capsule widths (L_1-L_4) for G_W and G_{10}-G_{12} were statistically similar for males and females. L_5, however, was significantly different in the capsule headcapsule width of both sexes ($p<0.001$, note appendix Table A.1). G_W had larger values of head capsule widths at L_5 ($p< 0.01$ at 1.23 ± 0.02mm and 1.25 ± 0.02mm for males and females respectively) compared to G_{10}-G_{12} (all $\pm$ = 95% confidence intervals).

Only 5 instars have been recorded in 12 generations of successive rearing of *C. jactatana*. It therefore appears that 5 instars is a determinate character for the species. The total duration of L_5 averaged 10.5 ± 0.5 days for both sexes and comprised of an active feeding phase of 4.3 ± 0.6 and 4.7 ± 0.5 days and a quiescent pre-pupal period of 4.7 ± 0.3 and 5.8 ± 0.8 days for males and females respectively). L_5 duration was not affected by number of generations of laboratory rearing. The pre-pupal period for G_W was significantly longer ($p<0.001$) by one day.

Unlike in *M. sexta* (Nijhout and Williams, 1974a; Jones *et*

al. as quoted by Bhaskaran, 1980; Safranek and Williams, 1984a),
starvation of L_5 produced neither supernumerary instars nor larval-
pupal intermediates. Those larvae transferred onto artificial diet
after 20 days of starvation developed and pupated successfully
although at lower pupal weights. The starvation regimen gave evidence
supporting the hypothesis that metamorphosis occurred only after the
attainment of a critical size in the final instar. The L_{CW} for *C.*
jactatana was 29.0 and 36.4mg for males and females of 1.18mm and
1.20mm headcapsule width respectively in G_{10}. L_{CW} was stable for the
three laboratory generations and G_W examined, occurring within a
range of 28.4-29.4mg and 35.8-36.4mg for males and females
respectively. Larvae could be starved for 27-30 days before death
occurred.

Figure 3.2 is a plot of daily larval wet weight from moult
into L_5 to pupation in G_{10} and G_W. Each larva weighed showed a
definite peak at 4-6 days. The peak is hereafter referred to as the
larval maximum weight (L_{MW}) and marks the onset of the pre-pupal
period. There were no discernible morphological markers that could
be associated with a second release of PTTH and subsequent surge in
ecdysterone. G_W had higher increase in daily weight than G_{10}-G_{12}.
The L_{MW} for both sexes in G_W were also higher (by 2.2mg for males
and 8.5mg for females) than for G_{10}. The difference between L_{MW} and
P_W for G_{10} was 11.8mg and 12.2mg for males and females respectively
(29.5 $\pm$3.3% for males and 24.8 $\pm$1.8% for females). Decrease in

weight from L_{MW} to P_W was not significantly different for G_{11}-G_{12} and G_W, remaining relatively constant at about 30% for males and 25% for females. Adult weights averaged 52.3 ±4.4% of the male L_{MW} and 62.4 ±1.7% of female L_{MW} for G_{10}. The percentage decrease from L_{MW} to A_W for G_{11}-G_{12} and G_W were not significantly different remaining at about 50% for males and 40% for females. A decrease of 15% and 10% in pupal weight was due to pupal case (meconium) in males and females respectively. Latent feeding periods averaged 3.4 ±0.7 and 4.8 ±0.5 days for males and females respectively in G_{10}-G_{12} and G_W. There was a direct correlation between the total duration L_5 and P_W (r=+0.48), and between the latent feeding period and P_W (r=+0.66) in G_{10}. There was no correlation between the latent feeding period and adult longevity or pre-oviposition period. A correlation coefficient of +0.2 was found between duration of latent period and the oviposition period in all generations tested.

Figure 3.3 is a plot of pupal weights versus fecundity of adults derived from L_5 starved at various weights (results were pooled from G_{10}-G_{12} due to a low incidence of mating). The regression equation is y=-160+7.83x, where y is the fecundity and x the pupal weight. The regression equation explains for 40.9% of the variation in y and is significant (p<0.01). No adult from a pupa weighing less than 31.1mg (19.1mg adult weight) laid any eggs.

3.5 DISCUSSION

3.5.1 *The larval critical weight (L_{CW})*

The larval critical weight (L_{CW}) is defined as the weight above which, normal processes of pupation (wandering, spinning, lightening of colour of the integument) occur, whereas starvation below that weight does not lead to the initiation of pupation processes or subsequent ecdysis. By deduction, L_{CW} is the trigger for the release of corpora allata-inhibiting factor which stops the secretion of juvenile hormone (Nijhout, 1975; Williams, 1975; Jones *et al.* 1981). L_{CW} is not an absolute value for a population of an insect species (contrary to Safranek and William (1984a 'absolute threshold'), but rather it is related to L_5 size (estimated from head capsule widths). Correlation between L_{CW} and head capsule width in *C. jactatana* was high (r=+0.98). Head capsule widths are directly related to the size of the penultimate instar (Jones *et al.* 1981, observations on *Trichoplusia ni*). As L_{CW} in *C. jactatana* was found to be relatively constant, I propose that, that L_{CW} is a fixed value and descriptive for equal sized members of a species just as the mean head capsule width is descriptive of size within a insect species.

3.5.2 *The Larval maximum weight (L_{MW})*

The L_{MW} may be defined as the highest level of weight that a

larva can attain. The L_{MW} peak corresponds to the first release of PTTH from the brain after total decay of the juvenile hormone (note Nijhout and Williams, 1974a; Williams, 1975; Jones *et al.* 1981). L_{MW} signals the onset of the pre-pupal phase. In *C. jactatana*, L_5 larvae stop feeding, wander to the tops of rearing containers, and initiate spinning. There is also some lightening of colour, the dorsal vessel and gut purge becomes progressively evident. In other insects, attainment of L_{MW} is the onset of pre-pupal morphological markers such as pale green colouration in *T. ni*, Jones *et al.* (1981) and negative phototaxis leading to digging into soil in *M. sexta*, Nijhout and Williams, (1974b). The higher values in pupal and adult weights in G_W are perhaps because of the sudden impact diet of high nutrition and a predator free situation.

Figure 3.4 represents sequential model of wet weight changes in L_5 of *C. jactatana* expressed as percentage of L_{MW}. L_{MW} is proposed as a population characteristic for a given species that is dependent on the initial size of L_5, environmental factors and nutrition. The period between day 0 and achievement of L_{CW} is described as the pre-L_{CW} period (Figure 3.1). In *C. jactatana* the duration is about 2.0 ±0.3 days for both males and females. The period between day 0 and L_{MW} (pre-L_{MW} period) is the active feeding phase and about 4.3 ±0.4 days for both males and females. The only apparent relationship between L_{CW} and L_{MW} in G_{10}-G_{12} and G_W, was that L_{CW} was consistently about 75% of the mean L_{MW} for both sexes. The

latent feeding period is the period between attainment of L_{CW} and L_{MW}. There is a gradual (4-7 days) decrease of weight from L_{MW} to pupa during the pre-pupal period. Weight decreases further during the pupal period to give the adult weight. L_{CW} is important as it ensures that regardless of the different growth rates for different larvae, pupation occurs only when final instar larvae have reached the 'required' size. 'Required' size means able to produce a pupa that will emerge to give a functional adult. A L_{CW}, therefore, may be regarded as a mechanism ensuring that the subsequent 'steps' in decrease in weight are crossed to leave a functional adult. The sexual dimorphic nature in L_{CW}, L_{MW}, pupal and adult weights is probably due to the different requirements for the sexes in reproduction. Relationship between L_{MW}, pupal and adult weight can be represented as:-

Pupal weight (P_W) = L_{MW} - (L_{MW} X D_P)

Adult weight (A_W) = L_{MW} - (L_{MW} X D_A)

where D_P = a constant of weight decrease from L_{MW} to P_W

D_A = a constant of weight decrease from L_{MW} to A_W

For *C. jactatana*, D_P is 0.3 and 0.25 and D_A is 0.6 and 0.5 for males and females respectively. All durations before L_{MW} are under the direct influence of environmental factors and quality of nutrition. Environmental factors (e.g. temperature) will speed or slow the post-L_{MW} developmental durations by affecting the rate of

enzyme and hormonal action.

3.5.3 L_{CW}, P_W, A_W and reproductive performance

Prediction of pupal weight from the female L_{CW} value for *C. jactatana* is:-

$$L_{CW} - (L_{CW} \times D_{P}\female) = 36.0 - (36.0 \times 0.25) = 27.0mg$$

A value of 27.0mg may be described as the female pupal critical weight (P_{CW}) or the lower limit of female pupal performance. Using the male L_{CW} value gives the male P_{CW} as 20.3mg. Similarly, a predicted adult weight value from female L_{CW} is:-

$$L_{CW} - (L_{CW} \times D_{A}\female) = 36.0 - (36.0 \times 0.5) = 18.0mg$$

The female adult critical weight (A_{CW}) or the lower limit of female adult performance is 18.0mg. A value of 11.6mg is derived from using the male P_{CW}. Theoretically, medium sized larvae cannot pupate at weights below P_{CW} or eclose below the A_{CW}. Incorporating the female P_{CW} into the regression equation gives a fecundity of 52 eggs/female. This, theoretically, is the lowest fecundity possible for medium sized *C. jactatana*. An increase of 1mg above the female P_{CW} leads to an increase of about 8 eggs in fecundity. In other words, when female P_W is equal to P_{CW} (27.0 mg), the number of fertile eggs laid would be 52. The smallest adult in Figure 3.3 had

a pupal weight of 31.1mg and a fecundity 96 eggs. This finding confirms the prediction of Slansky and Scriber (1985), who proposed that L_{CW} is "the minimal weight for pupation" which allows for "production of a functional adult".

The number of eggs displayed in Figure 3.3 actually correspond to realised fecundity rather than potential fecundity. Insect fecundity is greatly influenced by the number of ovarioles. The reproductive performance can be more accurately arrived at if ovariole count for female is correlated with pupal or adult weight. Realised fecundity here contains residual uncertainties and errors pertaining to other mating sources (e.g. success in spermatophore transfer, sperm fertility). Gunn and Gatehouse (1985) found a positive correlation between moth weight and fecundity. Wagner *et al.* (1987) have shown that fresh pupal mass was a precise predictor variable for potential fecundity. Other variables used were pupal abdomen width and dry mass of adults. Vogt and Walker, (1987) reported that the potential fecundity (number of ovarioles/ovarian cycle) of the female bush fly *Musca vetustissima* was directly related to fly size (headwidth). Potential performance for males may be expressed in terms of sperm production. Considering eclosion of different sized pupae (pooled from G_{10}-G_{12}), it was found that there was greater likelihood (80 %) of incomplete eclosion or wing deformity in adults from pupae within +5mg of the P_{CW} for both sexes than in any other P_W category.

There is a relationship between the adjustment of L_{CW} in relation to L_{MW} in different species and their lifestyle. Slansky and Scriber (1985) discussed the selective advantage of a low L_{CW} in insects with ephemeral diets and habitats or those developing in crowded conditions (e.g. L_{CW} is only 12% of normal dry weight in *Drosophila*, Beadle *et al.* (1938), as quoted by Slansky and Scriber, 1985.). Such insects also have relatively shorter duration in the latent feeding period. Univoltine species diapausing as pupae have high L_{CW} and longer latent periods (in the silkworm 60% of the final instar is spent in active feeding (Lees, 1955) so as to accumulate enough nutrients and metabolic reserves. The fact that *C. jactatana* has an L_{CW} of about 75% of the L_{MW} most likely reflects the long pre-pupal period the larva has to undergo (4-7 days). Why the pre-pupal period is long in *C. jactatana* is not clear.

3.5.4 Pupal and adult indices of quality

P_{CW} and A_{CW} are useful as descriptive indices of insect quality for both male and female insects. If the L_{CW} for a population of insects is taken to be equal to that of the smallest individual member, quality indices can be derived as:-

Pupal quality index=P_W/P_{CW}

Adult quality index=A_W/A_{CW}

Thus, where the mean pupal or adult weight for the population is equal to critical weights of the smallest member, the indices would be 1. As the weights increase above the critical weights, the indices would climb to above 1, representing an increase in quality.

3.6 CONCLUSION

There is a direct relation between the latent feeding period and adult performance. The longer that L_5 spend in the latent period, the greater the weight above L_{CW} and P_{CW} attained and hence the greater is the adult quality index. The limit of the latent feeding period is the L_{MW}. It seems probable that pupal and adult quality can be improved by increasing either the L_{MW} (e.g by high dietary quality) or by lowering the D_A (e.g. shortening the pre-pupal or pupal periods since Wagner *et al.* (1987) observed that pupae having longer pupal periods gave smaller adults). Quality may also be improved if hormonal changes during the latent period are slowed such that feeding and assimilation remain unaffected (e.g by temperature). Increasing size of insects does not appear beneficial as there is a concomitant increase in L_{CW}.

Changes in wet weight of the final instar were used by Blakley and Jacobson (1960) to determine accurate onset of the pre-pupal period. Hinks and Byers (1976) used a similar method to catalogue the genus *Euxoa* into three larval aestivating classes. Roe

et al. (1982) developed cumulative growth curves for sugar cane borers. Wagner *et al.* (1987) studied only weight changes after pupation. This paper is the first report linking performance and quality to the process of metamorphosis, especially L_{CW} of the final instar. Further work needs to be done to determine to what extent the sequential model for *C. jactatana* presented in Figure 3.4 is applicable to other holometabolous insects. The indices of pupal and adult quality demonstrate how P_W and A_W can be described in terms of their critical weights and in reference to a base value (L_{CW}). To evaluate the application of critical weights in the area of overall insect quality needs further results from diverse experimentation such as pheromone production, attractancy, mating propensity flight capability and startle responses.

Figure 3.1: Summary of events in the final instar

larva leading to pupation

(See Chapter 3 for details)

Moult into the final instar

Pre-L_{CW} period

Stops secretion of allatropin,
Starts secretion of allatohibin
Stops CA secreting JH ⟶ ⟵ Larval critical weight (L_{CW})

Decay of JH ⟶ Latent feeding period

First release of PTTH ⟶ ⟵ Larval maximum weight (L_{MW})
Induces prothoracic gland to secrete ecdysone
Converted to ecdysterone
Larva stops feeding, begins to wander

Pre-pupal period

Second release of PTTH ⟶ ⟵ Cuticle synthesis
Ocellar pigment retraction
Larval ecdysis

Pupation

Figure 3.2: Changes in wet weight of the final instar

larvae of *C. jactatana*

(n= 11 for G_{10} male

19 for G_{10} female

14 for G_W male

16 for G_W female
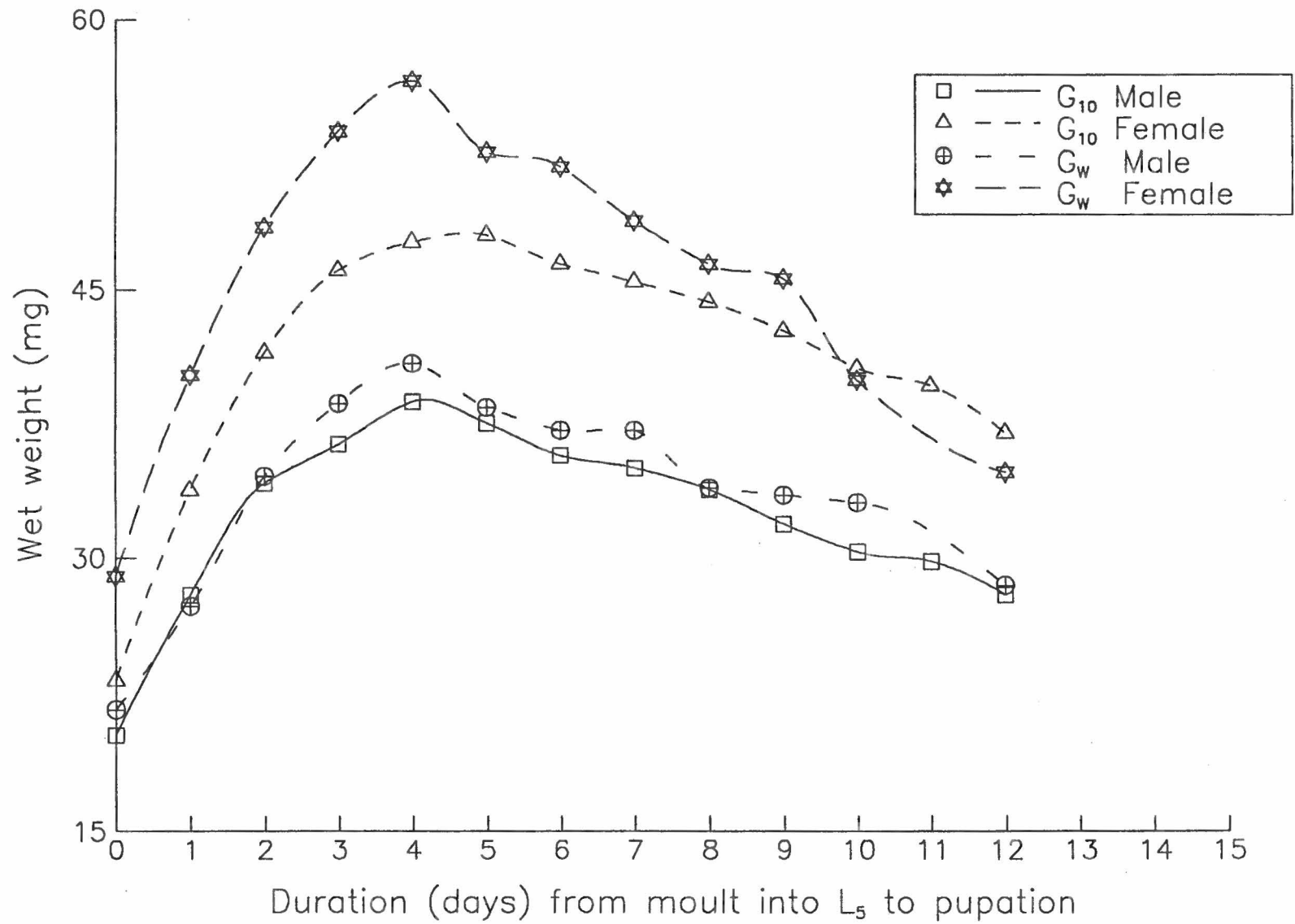
Figure 3.3: Relation between pupal weight and

fecundity in *C. jactatana*

(Based on 60 females from 3 generations)

REGRESSION
Fecundity=-160+7.83Pupal weight
r^2=0.409
t=5.20 DF=39
P<0.01
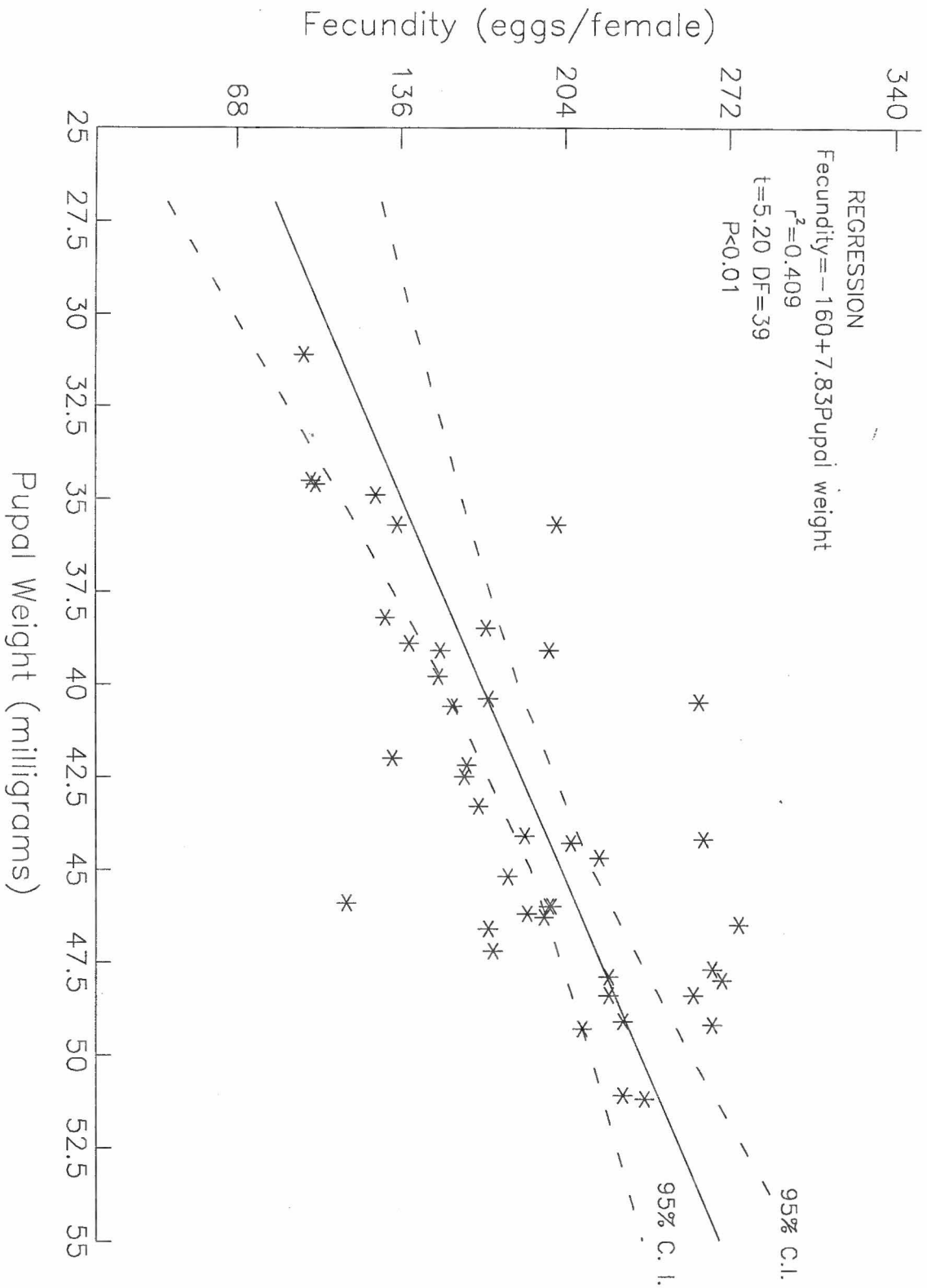
Figure 3.4: Sequential model of weight changes from

the final instar to adult *C. jactatana*

(See Chapter 3 for details)
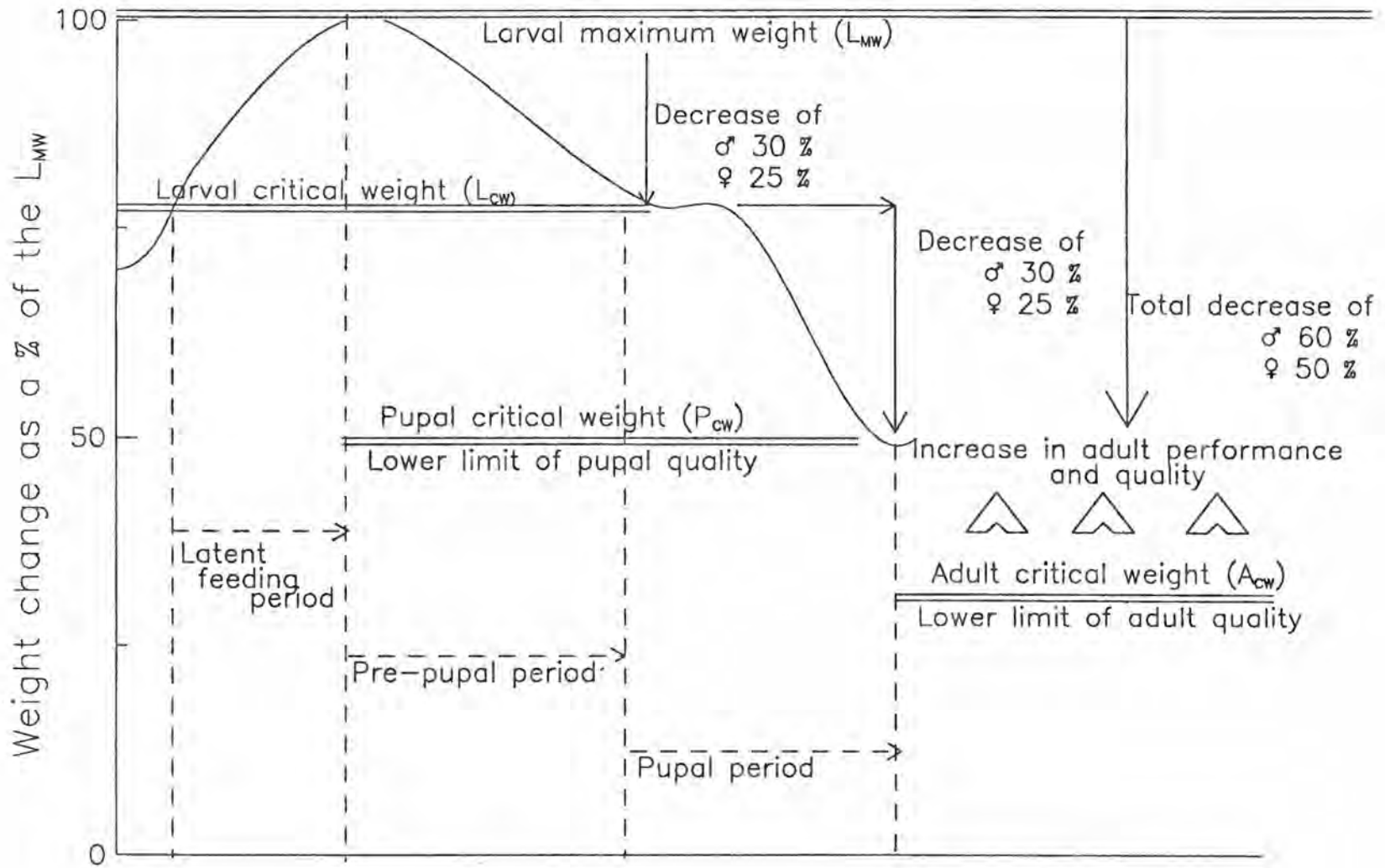
Figure axes:
- Y-axis: Weight change as a % of the L_{MW} (0, 50, 100)
- X-axis: Duration (days) from L_5 moult to adult eclosion

Labels:
- Larval maximum weight (L_{MW})
- Larval critical weight (L_{CW})
- Decrease of ♂ 30 % ♀ 25 %
- Decrease of ♂ 30 % ♀ 25 %
- Total decrease of ♂ 60 % ♀ 50 %
- Pupal critical weight (P_{CW})
- Lower limit of pupal quality
- Increase in adult performance and quality
- Adult critical weight (A_{CW})
- Lower limit of adult quality
- Latent feeding period
- Pre-pupal period
- Pupal period

CHAPTER / 4

THE RELATIONSHIP BETWEEN LARVAL CRITICAL WEIGHT, LATENT FEEDING PERIOD AND DIET QUALITY IN THE LARVAL METAMORPHOSIS OF *'Cnephasia'* *jactatana*

4.1 ABSTRACT

The lowest weight at which a final instar larva of the tortricid leafroller,*'Cnephasia'* *jactatana* initiates pupation (L_{CW}) was unaffected by diet quality. Use of a non-nutritive artificial 'diet' indicated that the larval-pupal metamorphosis was likely triggered mechanically by stretch receptors and therefore related to a fixed quantity of food ingested rather than nutrition. A final instar larva was not able to moult until it ingested and assimilated the quantity of food required to attain the L_{CW}. Diet quality was of prime importance during the latent feeding period (period between L_{CW} and the larval maximum weight, L_{MW}) in increasing the level of the L_{MW}. The decrease in weight from L_{MW} to pupal weight was dependent on diet quality; inadequate diet had higher values than diets of optimum quality. Weight decrease from pupa to adult was not significant under the different diets tested. Diet quality during the latent feeding period has direct implications on pupal and adult quality. The application of information gathered is demonstrated for

the rational selection, evaluation and improvement of artificial diets.

4.2 INTRODUCTION

The quantity and quality of food consumed and utilized by an insect larva determines such factors as rate of development, body weight and dispersal ability. Adult performance is directly dependent on larval nutrition as Hagen *et al.* (1984) and Slansky and Scriber (1985) concluded. In holometabolous insects, nutrient accumulation during the final instar can account for over 75% of the total food ingested during the larval stage (Waldabauer, 1968; Scriber and Slansky, 1981). It is likely therefore, that adult quality is closely linked to weight gain in the final larval instar.

In a recent paper (see chapter 3, especially Figure 3.3), it was shown that there was a consistent proportionate decrease in weight of about 30% for males and 25% for females from the highest weight that was attained during the final instar (larval maximum weight, L_{MW}) to the pupal weight of the leafroller '*Cnephasia*' *jactatana* (Walker) (Lepidoptera: Tortricidae). There was also a consistent proportionate weight decrease of 50% and 40% from pupa to adult for males and females respectively. Hence, the total weight decrease from L_{MW} was described by the constant D_A and was 0.6 and 0.5 of the L_{MW} for males and females respectively. It was proposed

that adult performance such as reproduction, could be improved if the level of the L_{MW} was increased by enhancing diet consumption and/or improving the diet quality during the latent feeding period (i.e. the period between achieving a larval critical weight for the final moult, L_{CW}, and attaining the L_{MW} (see Slansky and Scriber, 1985).

The trigger to the final moulting cycle in several insects is not entirely dependent on nutrition. For example, *Rhodnius prolixus* has been shown to depend on large blood meals to sufficiently stretch abdominal muscles (Wigglesworth, 1934). Beckel and Friend (1964) and later Anwyl (1972) confirmed that the initiation of pupation in *R. prolixus* was purely mechanical. Nijhout (1979, 1981), using saline injections and air inflation, demonstrated that moulting in the milkweed bug, *Oncopeltus fasciatus*, was triggered by stimulation of abdominal stretch receptors. To date, however, in contrast to the above hemipteran examples, the role of nutrition has not been clarified in the metamorphosis of Lepidoptera.

This paper set out to:-

1) Investigates role of nutrition in the processes that lead to pupation in *C. jactatana*.

2) Determine what the effect of diet quality during the latent feeding period was on adult performance.

Information gathered together with pupation rate, pupal and adult sex synchronism are demonstrated as useful for the rational

selection and evaluation of artificial diets.

4.3 MATERIALS AND METHODS

Fifth instar (L_5) larvae obtained either from a laboratory population maintained for eleven generations on a general purpose diet (GPD, Singh, 1983; appendix Table A.4) or from a population maintained for six generations on a sheep nut-bean based diet (SBD, see appendix Table A.5 for diet composition and appendix A.1 for rearing methods). To determine whether L_{CW} was influenced by diet, medium sized L_5 larvae (head capsule width 1.18-1.20mm; n=30 in each case) of various weights were selected from both diets after feeding for 12-24 hours following the moult into L_5 (day 0), then starved for 20 days. Larvae failing to pupate were transferred back to their original diet until pupation when sex was determined. Eclosing adults were sexed and weighed using a Shimadzu electronic balance (AEL-160) with an accuracy of ±0.1mg.

To ascertain whether nutrition was a factor in triggering pupation, 60 L_5 larvae of medium size were transferred on day 0 from GPD diet and placed in plastic vials (40 X 100mm) containing 1.5g of a non-nutritive 'diet'. The 'diet' contained cellulose powder (13%), agar (5%), water (80%) and sucrose (2%) added as a phagostimulant.

To investigate the effect of diet quality during the latent feeding period, 60 medium sized L_5 larvae were transferred from the original GPD diet on day 0 to plastic vials containing one of three diets: a) a low quality diet (25% GPD); b) 100% GPD; and c) SBD. To confirm that past larval diet history had no effect on the latent feeding period, other similar-sized L_5 larvae (n=30) reared on SBD diet from L_1-L_4 were fed on GPD diet. Experiments were carried out at $20 \pm 1°C$, $75 \pm 5\%RH$ with an LD of 18:6. Reproductive performance was evaluated at $15 \pm 1°C$, $75 \pm 5\%RH$ with an LD of 16:8. All larvae were individually weighed at 24hr intervals. Results were subjected to one way analysis of variance and t-tests using Minitab statistical package (Ryan *et al.* 1985).

4.4 RESULTS

4.4.1 L_{CW} and diet quality

Starving L_5 larvae which had been reared on either a bean based diet (SBD) or a synthetic diet (GPD) had no significant effect on the L_{CW}. L_{CW} for medium sized larvae was 29.1 ± 1.1mg for males and 36.0 ± 1.5mg for females fed on GPD diet compared to 30.5 ± 0.8mg for males and 37.7 ± 1.2mg for females fed on SBD diet (all $\pm = 95\%$ confidence intervals).

4.4.2 Diet quality as a trigger for pupation

Larvae fed on the non-nutritive 'diet' fell into two

categories; a) larvae that did not attain more than 30mg on feeding and did not initiate pupation (lightening of colour, dorsal vessel becoming apparent, gut purge, wandering and spinning at the onset of the pre-pupal stage) although were alive for over 45 days (77%); and b) larvae that attained weights above 30mg and initiated pupation (23%). Most larvae that initiated pupation died during the pre-pupal period and only 5 larvae pupated successfully, all being males. None of the pupae eclosed. Analysing the data for size (head capsule width) revealed that the initiation of pupation was not related to body size.

4.4.3 Effect of diet quality during the latent feeding period

Figure 4.1(a-c) shows cumulative daily wet weight gain and Table 4.1 the effect of diet quality on L_5 larvae fed on the three diets. Wet weight gain was greater under SBD compared to 100% GPD and 25% GPD. The mean daily increase for SBD was 7.0 ±2.3mg and 8.8 ±2.0mg/day compared to 5.0 ±0.7mg and 6.5 ±1.5mg/day under 100% GPD; and 2.1 ±1.4mg and 2.5mg ±2.3/day under 25% GPD for males and females respectively. Previous diet history had no effect on L_5 as results from larvae that had been reared on SBD from L_1-L_4 were not significantly different to those reared on GPD. One way analysis of variance indicated a significant difference for pre-L_{CW} period at $p < 0.01$ for males (F=19.7, DF= 105). Female pre-L_{CW} period was not significant at any level. The duration of the active feeding (pre-L_{MW}) was not significant for the three diets, although the

pre-pupal period for 25% GPD was significantly longer at p<0.01 form the other two diets. Male latent feeding period was significant (F=24.8, DF=105) although only the female latent period under SBD was significant (P< 0.01) from both GPD diets. L_{MW} was significant at p<0.001 for both sexes (F= 35.2, DF=105 for males and F=44.1, DF=69 for females). Decrease in weight from L_{MW} to pupa (D_P) was significant at p< 0.01 for both sexes (F= 38.5, DF=101 for males and F= 28.4, DF= 58 for females). There were no significant differences at any level for the decrease from pupal to adult weight (D_A).

4.5 DISCUSSION

4.5.1 Diet quality in the final instar as a trigger for pupation

Results of the experiments suggest that a minimal weight is necessary to initiate pupation which is achieved by ingestion of a specific quantity of food. All larvae that attained a weight of 30mg began pupating while those that did not attain 30 mg failed to initiate pupation. A value of 30mg is about equal to the male L_{CW} of 29mg recorded for *C. jactatana* larvae of 1.18mm head capsule width (chapter 2). The bulk of the non-nutritive 'diet' consisted of cellulose which is not digestible by lepidopteran larvae (Dadd, 1985; Reinecke, 1985). Agar is a gelling agent of no known nutritional value (except probably for trace minerals, Vanderzant, 1974). The sucrose added might contribute as an energy source. Since some larvae were able to initiate pupation by feeding on the

'diet' alone confirms that nutrition is not an *a priori* necessity for the initiation of pupation. Larvae were offered the 'diet' as the only choice available. Quantity ingested depended wholly on individual larvae. Since 77% larvae did not initiate pupation even after 45 days on the 'diet' suggests that assimilation and growth must be important in attaining the L_{CW} for *C. jactatana*. Time spent in the instar is of no consequence to pupation since during the entire 45 days larvae were observed feeding and voiding fecal pellets.

It is likely, therefore, that unlike *Rhodnius prolixus* (Wigglesworth, 1934; Beckel and Friend, 1964), but like *Oncopeltus fasciatus* (Nijhout, 1979; 1981), some nutrition is crucial in the Pre-L_{CW} period of the final instar of *C. jactatana* to bring the larvae to a state of pupation competence. That is, L_5 larvae of *C. jactatana* are not of sufficient weight and development at the time of moult to L_5 and must therefore ingest more in order to 'satisfy' this minimal weight. The experiment would be more conclusive if some artificial means were used to force ingestion. Attempted methods of injection were not successful as larvae died immediately afterwards (attempts by Nijhout, 1981, were similarly unsuccessful using a saline injection method). Once larvae are competent to pupate, it is likely that mechanical mechanisms involving stretch receptors are responsible for the initiation of the pupation. Since some larvae actually attained L_{CW} and initiated pupation after ingesting the non-

nutritive diet tends to support this hypothesis. It would mean that it is unlikely that nutrition is of consequence in triggering the release of the brain stimulating factor that starts the pupation process (Bhaskaran *et al.* 1980; see also chapter 3). Only males successfully concluded pupation. The higher L_{CW} for females of about 36mg demands relatively more ingestion. No larva feeding for the 45 days attained a weight of 36mg.

Nijhout's (1981) work on *Manduca sexta* considered larval moult to be of two main types. Larvae identify the final instar by some form of allometry. At the final instar, however, larval metamorphosis is triggered by a critical weight. If assimilation is necessary during the pre-L_{CW} period, the L_{CW} must be tuned to assess competence in terms of assimilation. This contradicts the L_{CW}-mechanical related hypothesis, and raises the question of the nature of the mechanism that gauges assimilation in L_5 larvae. Larva are not able to ingest enough at 'will' to trigger pupation. Mortality during pupation identifies nutrition within L_5 as necessary for successful pupation. Nutritional inadequacy also explains the failure of the five male pupae to eclose. The observation that larvae reared on either SBD or GPD did not affect the L_{CW} for similar sized larvae supports the L_{CW}-mechanical stretch hypothesis. Similar sized larvae of whatever nutritional background would be expected to exhibit similar L_{CW}.

4.5.2 Diet quality during the latent feeding period

The larval critical weight, L_{CW}, for a population of an
insect species may be regarded as the minimum weight required by the
smallest member of the population to initiate pupation (see chapter
2). Using the L_{CW} of the smallest larvae to set the onset of the
latent feeding period for a group of larvae with a narrow range of
somatic size eases the statistical analysis. Moreover, it is
justified in setting the onset to the latent feeding period since the
difference between the L_{CW} of the smallest and the largest larva of
either sex in *C. jactatana* is less than 10mg, a weight that a larva
can ingest within one day.

In a previous discussion (chapter 3), the latent
feeding period was presented as a duration that depended on the
underlying hormonal events. The end of the latent period depends
upon the release of the prothoracicotropic hormone (PTTH, Nijhout
and Williams, 1974; Nijhout, 1981). Latent feeding period cannot be
'postponed' once the L_{CW} is attained as it depends on the half life
or decay of juvenile hormone. All other factors being equal, latent
feeding period can be regarded as a invariable stage between two
hormones as PTTH will not be released until the decay of juvenile
hormone.

In order not to compound diet effects, the three diets were
fed to L_5 larvae from day 0 and not only at the commencement of the

latent feeding period. The results indicate that the pre-L_{CW} for the three diets is dependent on diet type. Diet of low nutritional quality significantly lengthens the pre-L_{CW} period compared to high quality diet. This observation confirms that attainment of the L_{CW} depends in part on assimilation and growth. Where the diet is of poor quality, ingestion is slow because of low assimilation. It is probable that amount of food ingested is low because of size limitations.

The latent feeding period was affected by diet quality. Improving diet quality lengthened the latent feeding period significantly under GPD and SBD. Improving diet quality, however, shortened the pre-L_{CW} period. About 65% male and 38% female larvae attained the L_{CW} within the first day, the rest on the second day of feeding on SBD, compared to only 17% for both males and females attaining the L_{CW} within the first day under 25% GPD (59% male and 46% females attained the L_{CW} after three days). Since the overall diet type did not affect the pre-L_{MW} period, it would seem there is a indirect relationship between the duration of pre-L_{CW} period and the latent feeding period. Larvae had considerably longer latent periods when they achieved the L_{CW} faster and shorter latent feeding periods when they took longer to achieve the L_{CW}. The most plausible explanation for this relationship is some underlying nutrition-hormonal interaction of undetermined nature affected by diet quality. It has been shown, for example, that low doses of juvenoids fed to

final instar larvae prolong larval feeding and growth (Sláma, 1975; Sehnal, 1976; Ciemior *et al.* 1979).

Figure 4.1(a-c) demonstrates that daily total larval wet weight gain reached a maxima on day 4 for males fed both GPD concentrations. Females fed 25% GPD took one day longer than the females fed GPD. Daily larval wet weight gain for SBD reached its maxima on day 6 for both males and females. There was a distinct tapering in wet weight gain in the last day before the L_{MW} in all diets. Consumption of diet is not useful to increase the level of L_{MW} if it is not assimilated because body size is limiting without growth. It is proposed that in diets where assimilation and growth is high, L_{MW} will be high (L_{MW} is about 54% above the L_{CW} in larvae fed on SBD compared to 13% in larvae fed 25% GPD). During the pre-pupal period, all unassimilated gut contents are purged. D_P was significantly higher for 25% GPD compared to the other diets due to a higher percentage of gut purge unassimilated food material (dilution of GPD was done using agar to substitute the 75% reduction in nutrients). The relatively higher value of D_P for SBD is related to diet composition. GPD is a synthetic diet composed of mainly refined chemical substances while SBD contains unrefined plant substances that include lucerne meal and lima beans. More material is therefore present in SBD that cannot be assimilated (e.g. fibrous lignin).

Measure of the L_{MW} accounts for consumption, digestibility,

and efficiency of conversion in the final instar of *C. jactatana*. Weight gain above L_{CW} depends on the nutritional quality of the diet that will be assimilated. Diets are only useful to increase the L_{MW} if there is rapid ingestion coupled with assimilation and growth. Consumption in the latent feeding period contributes to weight increase when the diets have high utilization value, referred to as larval efficiency in converting ingested food into body matter, ECI. The efficiency by which a larva converts digested food into body matter is described by index ECD (Waldabauer, 1968). In other words, L_{MW} will be high when ECD and ECI are high. As D_A is relatively constant, performance of adults (and therefore quality) directly reflects on the levels of L_{MW}, ECI and ECD.

4.5.3 L_{CW}, L_{MW} and nutritional ecology

Growth, development and reproduction of insects are directly linked to quantity and quality of ingested food (Hagen et al. 1984). In a ecological context, the relation is complicated as food must be found, accepted and ingested. The quality of diet at the latent period will contribute to the overall quality of a population. While working on the southern green stink bug, *Nezara viridula*, Kester and Smith (1984) demonstrated conclusively that inadequate diets during the fifth and final stadium irreversibly affected growth, development, reproduction and flight capacity of adults. Slansky and Scriber (1985) have referred to the L_{CW} as the minimum weight required to give a functional adult. Larvae have to

accumulate reserves (anabolism) that adults require to live on
(catabolism). Ziegler, (1985) reviewed changes in activity of
glycogen phosphorylase regulating haemolymph sugar in *M. sexta*. The
regulation is linked to a brain released hormone. Early in
starvation, glycogen phosphorylase is activated to utilise haemolymph
sugar, but inactivated under prolonged starvation. Adults, however,
are purely catabolic, using all energy reserves in starvation.
Therefore, it is important for a final instar larva to accumulate as
much reserves as possible (high L_{MW}) to sustain adult life. In
conditions of inadequate nutrition, L_5 larvae pupate at the L_{CW}.
Where L_{CW} is not attained larvae will live on available reserves
searching for better nutrition. If nutrition is unavailable,
catabolic enzymes are inactivated and larvae starve to death. L_{CW} is
therefore all-or-none and not under 'choice' of final instar larva.
Subsequent factors after attainment of the L_{CW} are more complicated
involving environmental factors acting independently or
synergistically on the endocrine function (Keely, 1972).

The blood sucking *R. prolixus* offers a life style distinctly
different from the phytophagous *O. fasciatus* and *C. jactatana*).
Blood sucking Reduviidae live a precarious existence without
certainty of meals. The uncertainty in *R. prolixus* is compensated
for by engorging nine times it's body weight in one feeding when a
blood meal is available (Wigglesworth, 1934; Friend *et al.* 1965).
L_{CW} is therefore regulated to conform to the single meal. Plant food

is not as euphemeral for *O. fasciatus* or *C. jactatana* (8 plant species have been recorded for *C. jactatana*, Spiller *et al.* 1982), therefore the L_{CW} is regulated to a higher level needing a degree of assimilation.

4.5.4 Evaluation of artificial diets

Table 4.2 presents results of evaluation of artificial diets for *C. jactatana* based on the GPD as a standard diet. Prediction of female reproductive performance (fecundity) was based on an equation obtained from regressing fecundity on pupal weight of *C. jactatana* reared on GPD diet ($y=-160+7.83x$, $r^2=0.409$, $P<0.01$; see chapter 3). Quality index is calculated from the ratio of pupal weight to pupal critical weight (P_{CW}), where the P_{CW} is 20.3mg for males and 27.0mg for females. The pupal quality index is greater than 1 when the pupal weight is higher than the P_{CW} (see chapter 3 for a full discussion). Pupation rate index (R_P) is the ratio of the number of pupae formed by the first adult eclosion to the total pupae. When all pupae are formed by the first adult eclosion, R_P is 1. R_P is an indicator of the larval rate of development. Sex synchronism indices describe relative male and female coincidences of pupation (S_P) and eclosion (S_A). The indices are 1 when there is a male for each female pupating or eclosing and 0 when there is no coincidence (note chapter 2 for full equation).

In the diets evaluated, only SBD had higher reproductive

performance than predicted by a similar pupal weight of GPD. The pupal quality index was equal for both diets. The higher observed reproductive performance of SBD indicates a nutritional superiority of SBD over GPD. SBD contains brewers yeast which is rich in nucleotides and B vitamins. Brewers yeast has been quoted to have factors that stimulate amino acid assimilation and growth (Gilmour, 1961; Vanderzant, 1974). SBD has been successfully used for seven generations of laboratory rearing of *C. jactatana* (see Chapter 2 for detailed results). Bean diet (Shorey and Hale, 1965) had the highest pupal quality index although, the realised fecundity was far less than the predicted value. The low fecundity suggest that the quality of the bean diet is not optimal. The other diets evaluated had lower observed performance than their predicted values. The only diet that equaled GPD in rate of larval development (with R_P index of 1) was SBD. Synchronism indices were low in sheep nut diet (Thomas, 1968) because of the long larval development.

4.6 CONCLUSION

Nutrition is of no consequence in the initiation of pupation. It is likely that the L_{CW} is a mechanical trigger related to a fixed quantity of diet ingested. Ingestion must be accompanied by assimilation in *C. jactatana* for the L_{CW} to be effective. Diet quality is important during the latent feeding period to increase the level of L_{MW}, pupal and adult quality.

Table 4.1: Effect of diet quality on the final

instar of *C. jactatana*

* at 20 ±1°C, 75 ±5%RH

** mean «±» 95% confidence intervals

Rows followed by the same letter are not

significantly different (p>0.01)

		Artificial diet[*]		
		25% GPD	100% GPD	SBD
n	♂	32	39	34
	♀	24	20	26
Duration of L_a (days)	♂	14.9a[**] 13.9⟨±⟩15.9	11.9b 11.6⟨±⟩12.2	12.7c 12.5⟨±⟩12.9
	♀	13.8a 11.8⟨±⟩15.8	11.6a 11.2⟨±⟩12.0	13.5a 13.1⟨±⟩13.9
Pre-L_{aw} period (days)	♂	4.1a 3.0⟨±⟩5.2	2.0b 1.7⟨±⟩2.3	1.2c 1.0⟨±⟩1.3
	♀	2.5a 1.7⟨±⟩3.3	2.0a 1.7⟨±⟩2.3	1.5a 1.2⟨±⟩1.8
Pre-L_{aw} period (days)	♂	6.1a 5.2⟨±⟩7.0	5.2a 4.9⟨±⟩5.5	5.4a 5.0⟨±⟩5.8
	♀	5.6a 4.6⟨±⟩6.6	5.5a 5.2⟨±⟩5.8	5.2a 4.8⟨±⟩5.6
Pre-pupal period (days)	♂	8.7a 7.7⟨±⟩9.7	5.5b 5.1⟨±⟩5.9	5.5b 5.1⟨±⟩5.9
	♀	8.1a 6.2⟨±⟩10.0	4.9b 4.4⟨±⟩5.3	4.9b 4.4⟨±⟩5.4
Latent feeding period (days)	♂	2.4a 2.1⟨±⟩2.7	3.2b 2.9⟨±⟩3.5	4.1c 3.8⟨±⟩4.4
	♀	3.0a 2.4⟨±⟩3.6	3.7a 3.2⟨±⟩4.2	5.2b 4.8⟨±⟩5.6
L_{aw} (mg)	♂	33.4a 31.5⟨±⟩35.3	38.9b 37.2⟨±⟩40.6	44.3c 42.3⟨±⟩46.3
	♀	40.1a 38.1⟨±⟩42.1	47.1b 44.9⟨±⟩49.3	56.1c 53.0⟨±⟩59.1
D_p (%)	♂	47.1a 42.8⟨±⟩51.4	29.0b 26.4⟨±⟩31.6	33.7c 31.6⟨±⟩35.8
	♀	42.6a 37.7⟨±⟩47.5	24.8b 23.0⟨±⟩26.6	32.1c 30.2⟨±⟩34.0
D_a (%)	♂	49.9a 43.5⟨±⟩56.3	47.7a 43.4⟨±⟩52.0	46.8a 44.1⟨±⟩49.5
	♀	37.1a 35.2⟨±⟩39.0	37.6a 35.9⟨±⟩39.3	38.1a 36.5⟨±⟩39.7

Table 4.2: Diets evaluated for the rearing of *C.*

jactatana

* at 18 ±1°C, 75 ±5%RH, Reproductive

performance at 15 ±1°C, 75 ±5%RH

** mean «±» 95% confidence intervals

Rows followed by the same letter are not

significantly different (p>0.01)

		Artificial diet[a]						
		GPD Singh (1983)	25% GPD	GPD/Acmena Clare and Singh (1988)	Raspberry leaf Hobson and Singh (1987)	Sheep nut Thomas (1968)	Bean Shorey and Hale (1965)	SBD
n	♂	51	27	42	61	41	46	36
	♀	43	34	40	45	29	38	39
Mean larval period (days)	♂	33.9a[**] 33.4≪±≫34.4	49.3b 47.8≪±≫52.3	44.0c 42.2≪±≫45.8	47.7c 45.7≪±≫49.7	60.7d 58.7≪±≫62.7	34.9a 33.5≪±≫36.3	38.9e 37.8≪±≫40.0
	♀	35.4a 34.6≪±≫36.2	50.5b 49.3≪±≫51.7	46.4c 44.4≪±≫48.4	48.0c 45.9≪±≫50.1	64.1d 60.8≪±≫67.4	37.0a 36.0≪±≫38.0	40.5e 39.3≪±≫41.7
Mean pupal weights (mg)	♂	30.3a 29.3≪±≫31.3	24.4b 23.3≪±≫25.5	29.7a 28.2≪±≫31.2	24.9b 24.2≪±≫25.6	24.8b 23.8≪±≫25.8	32.4c 31.7≪±≫33.1	30.2a 29.4≪±≫31.0
	♀	41.3a 39.7≪±≫42.9	30.8b 29.8≪±≫31.8	38.7a 37.0≪±≫40.4	32.5b 31.4≪±≫ 33.6	30.8b 29.1≪±≫32.5	43.9a 42.7≪±≫45.1	42.1a 41.3≪±≫42.9
Pupal quality index	♂	1.49a 1.44≪±≫1.54	1.20b 1.15≪±≫1.25	1.46a 1.39≪±≫1.53	1.23b 1.19≪±≫1.27	1.22b 1.17≪±≫1.27	1.60c 1.56≪±≫1.64	1.49a 1.45≪±≫1.53
	♀	1.52a 1.47≪±≫1.57	1.14b 1.10≪±≫1.18	1.43a 1.37≪±≫1.49	1.20b 1.16≪±≫1.24	1.14b 1.08≪±≫1.20	1.63c 1.58≪±≫1.68	1.55a 1.53≪±≫1.57
Pupation rate index (R_P)		1.0	0.61	0.84	0.89	0.79	0.91	1.0
Pupal synchronism index (S_P)		0.64	0.66	0.63	0.62	0.40	0.62	0.59
Adult synchronism index (S_A)		0.70	0.72	0.50	0.53	0.35	0.68	0.62
Predicted reproductive performance (eggs/ female)		163.4 150.8≪±≫176.0	81.2 73.3≪±≫89.1	143.0 129.7≪±≫156.3	94.5 85.9≪±≫103.1	81.2 67.9≪±≫94.5	183.7 174.3≪±≫193.1	169.6 163.4≪±≫175.8
observed reproductive performance (eggs/ female)		211.6a 132.4≪±≫290.6	75.1b 19.2≪±≫131.0	48.6b 20.4≪±≫76.8	64.1b 22.5≪±≫105.7	86.1b 37.6≪±≫134.6	99.6b 55.6≪±≫143.6	261.1a 211.5≪±≫310.7

Figure 4.1: Cumulative daily wet weight gain in the

final instar of *C. jactatana*

a) 25 % GPD

Vertical bars represent 95% confidence intervals

(n= 32 for male

24 for female)

Figure caption: Wet weight increase (mg) versus Days from L_5 moult to larval maximum weight (L_{MW}). Male wet weight gain and Female wet weight gain.
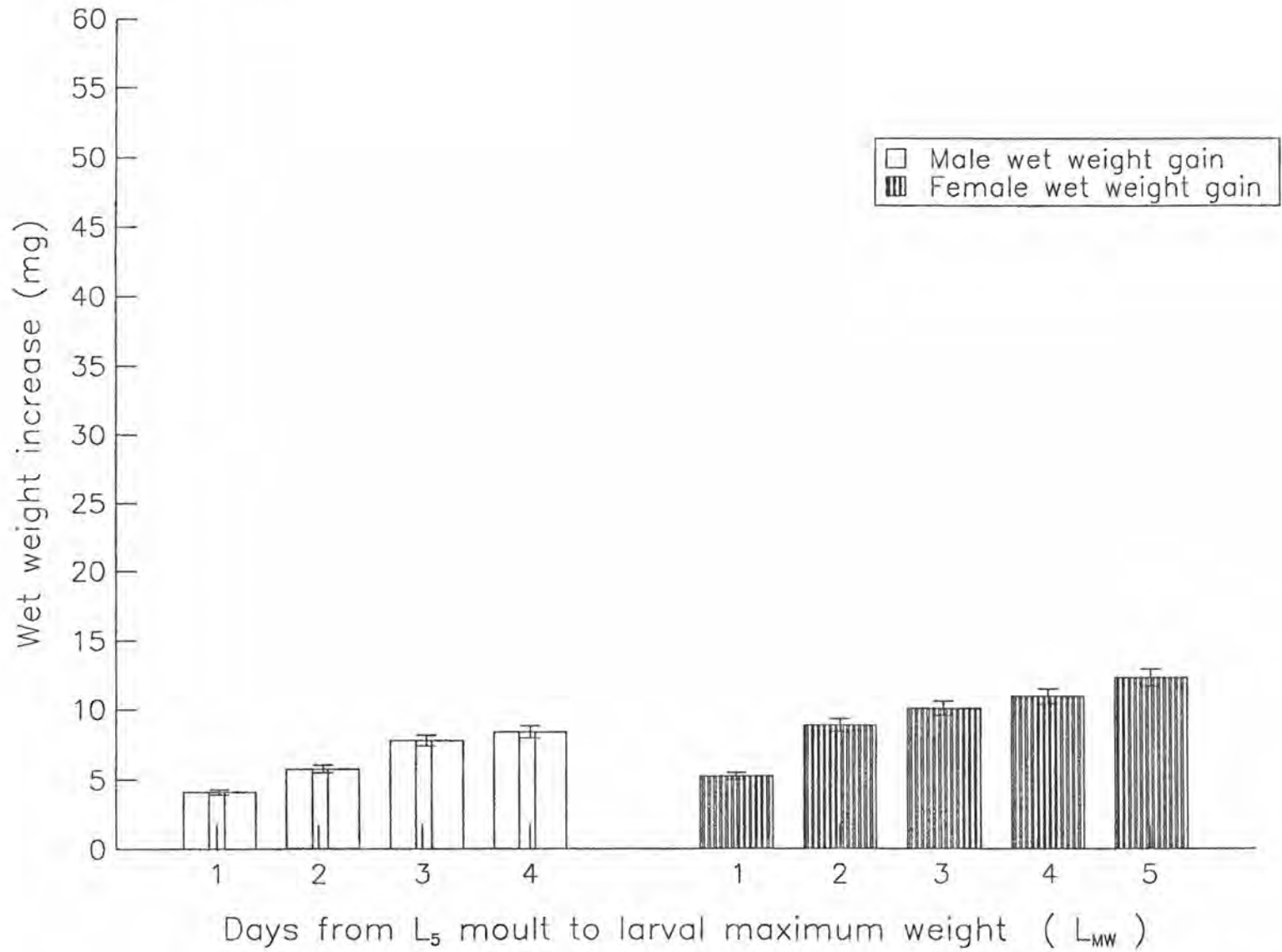
Figure 4.1: Cumulative daily wet weight gain in the

final instar of *C. jactatana*

b) 100 % GPD

Vertical bars represent 95% confidence intervals

(n= 39 for male

20 for female)

Wet weight increase (mg) versus Days from L_5 moult to larval maximum weight (L_{MW})

Legend:
☐ Male wet weight gain
▥ Female wet weight gain
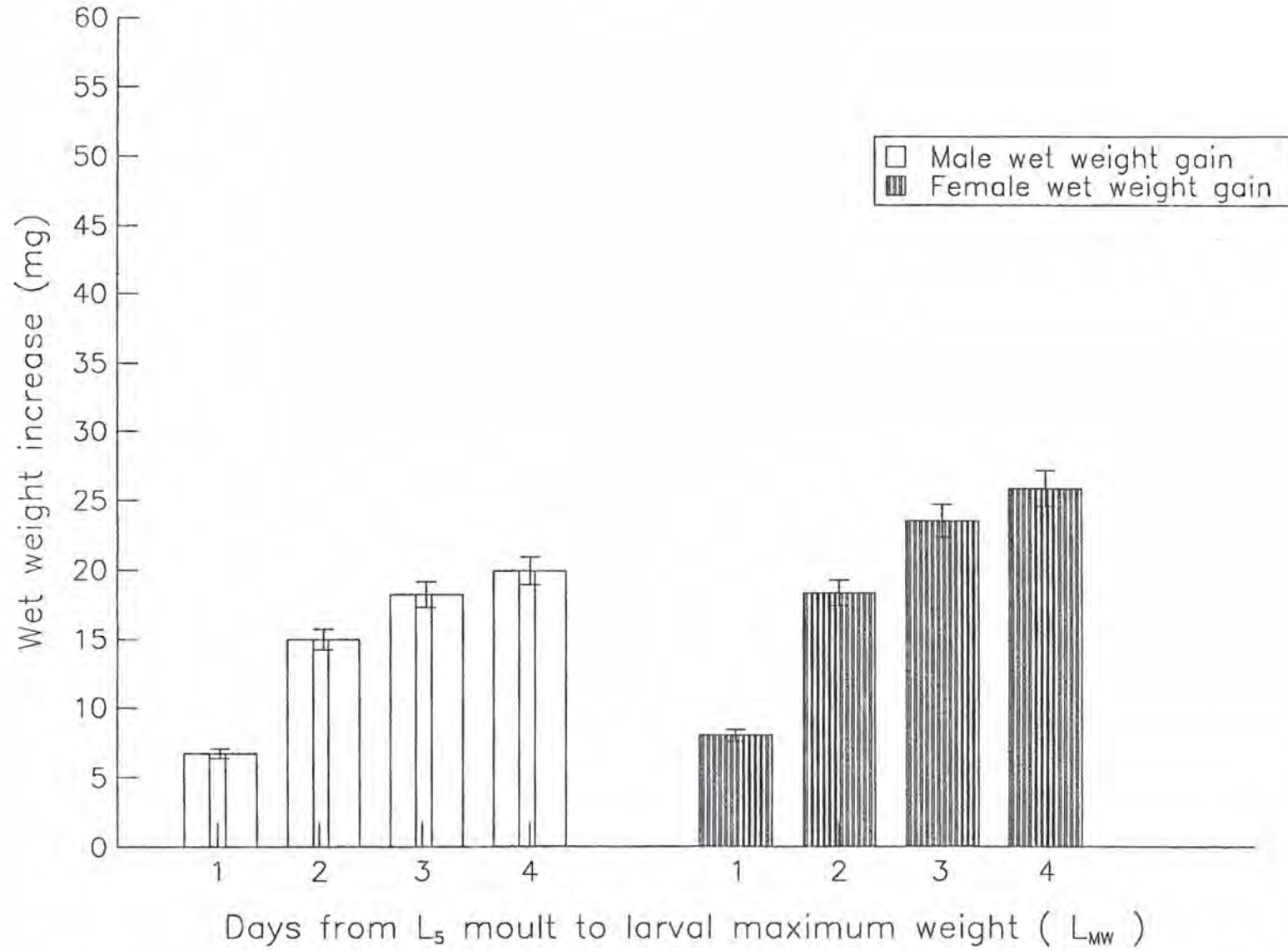
Figure 4.1: Cumulative daily wet weight gain in the

final instar of *C. jactatana*

c) SBD

Vertical bars represent 95% confidence intervals

(n= 34 for male

26 for female)

Days from L_5 moult to larval maximum weight (L_{MW})
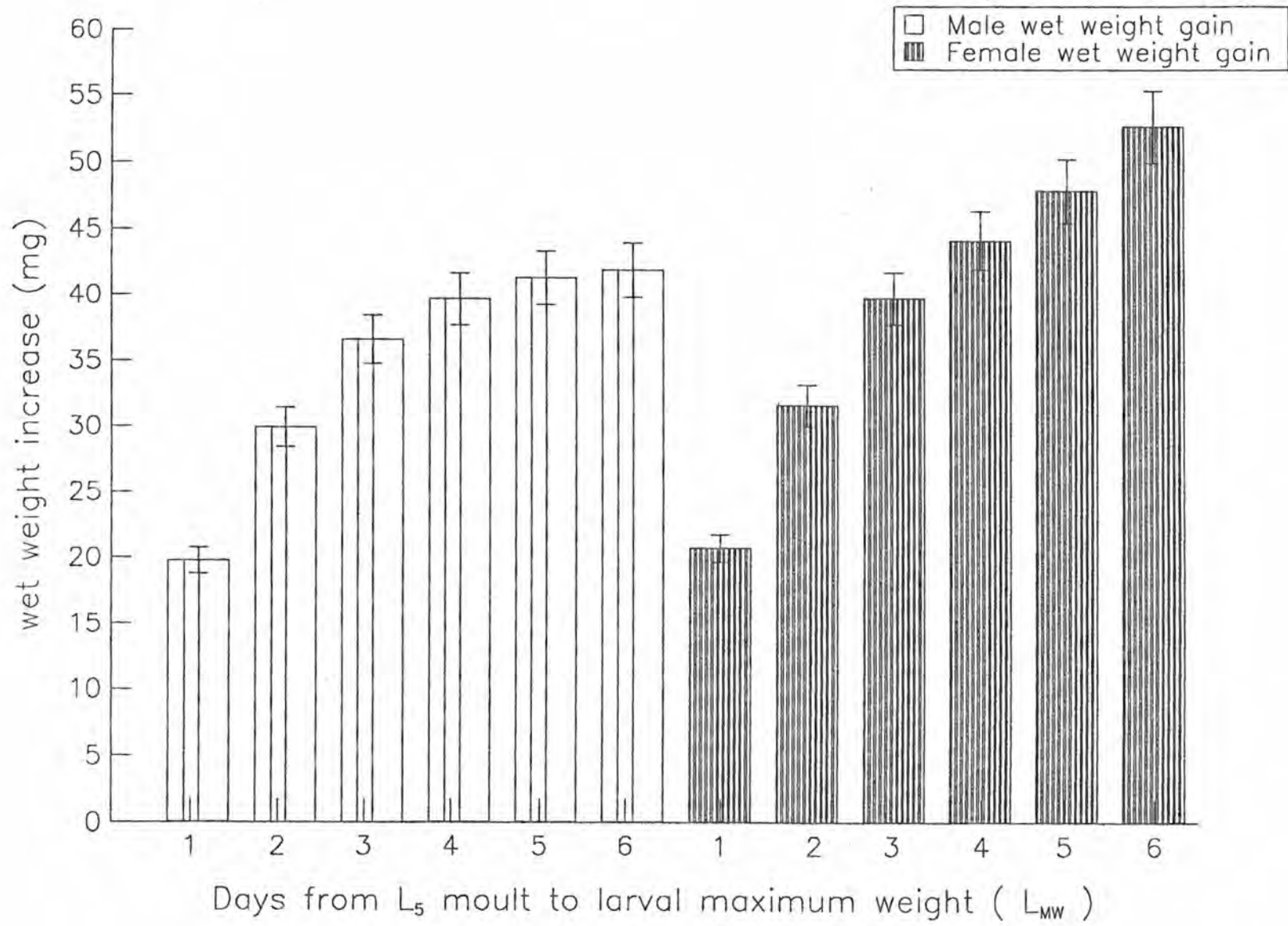
CHAPTER / 5

THE EFFECT OF THREE ABIOTIC FACTORS ON THE LARVAL CRITICAL WEIGHT, LATENT FEEDING PERIOD, LARVAL MAXIMUM WEIGHT AND FECUNDITY OF

'*Cnephasia*' *jactatana* (Walker) (Lepidoptera: Tortricidae)

I. CONSTANT TEMPERATURE

5.1 ABSTRACT

The larval critical weight (L_{CW}) is not dependent on temperature. Three constant temperatures tested (25°, 20° and 15° C) had no significant effect on the L_{CW} of final instar larvae of '*Cnephasia*' *jactatana*. The latent feeding period, however, was prolonged from 3 days at 25°C temperature to about 9 days at 15°C. The larval maximum weight (L_{MW}) increased from 21.3% and 23.8% of the L_{CW} at 25°C to 53.4% and 55.8% of the L_{CW} at 15°C for males and females respectively. Since drop in weight from L_{MW} to pupal weight (D_P) and drop from L_{MW} to adult weight (D_A) was not affected by temperature, lowering the rearing temperature led to higher pupal and adult weight probably due an overall increase of feeding and assimilation during the latent feeding period. Heavier females from 15°C were significantly more fecund than females from 20° or 25° C. Increase of the latent feeding period due to temperature is therefore important in improving reproductive performance and the overall quality of adult insects.

5.2 INTRODUCTION

Temperature has determinate action on insect development because their biochemical reactions are temperature sensitive. Most insects are ectothermic (gain heat from the environment) maintaining their body temperature close to ambient. The temperature range of activity for most biochemical processes, however, is small, and departure of insects from the optimum slows or speeds rate and performance of biochemical reactions. Prolonged exposure to temperatures exceeding the critical limits (Putman and Wratten, 1984; Hughes *et al.* 1984 see also chapter 2) lead to irreversible disorganization and destruction of metabolic pathways culminating in death (Hoffman, 1985).

Quality and performance in insects is dependent on weight gain from intake of nutritional diet (Kester and Smith, 1984; Slansky and Scriber, 1985; Engelmann, 1984; Bernays, 1985). Ratte (1985) reviewed the effect of temperature on insect size or weight. Some insects show a direct relationship between weight and temperature; increase in temperature results in heavier insects (e.g Douglas-fir tussock moth, *Orgyia pseudotsugata*, Beckwith, 1982); others decrease size with increase in temperature (e.g green cloverworm, *Platypena scabra*, Hammond *et al.* 1979) while other insects are seemingly not affected by temperature change (e.g. European corn borer, *Ostrinia nubilalis*, Beck, 1983).

Holo and hemimetabolous insects terminate growth by metamorphosis. Growth curves of the initial larval development of these insects are remarkably similar but vary during the final instar (Ratte, 1985). It has been shown that in several insects, the cue for pupation is the larval critical weight (L_{CW}) that a larva will attain in the final instar (Nijhout, 1975, 1979, 1981; Williams, 1980; Jones *et al.* 1981; Woodring, 1983). In the native New Zealand leafroller, '*Cnephasia' jactatana* the L_{CW} is about 29mg and 36mg for male and female fifth instar larvae (L_5) of headcapsule width of 1.18 and 1.20mm respectively (chapter 3). The period between attaining an L_{CW} and attaining the highest larval wet weight (Larval maximum weight, L_{MW}) is referred to as the latent feeding period (Nijhout and Williams, 1974a). The latent feeding period is related to the duration that the juvenile hormone takes to decay before the prothoracicotropic hormone (PTTH) is released signalling the onset of the pre-pupal phase (Jones *et al.* 1981; Nijhout, 1981, Bollenbacher and Granger, 1985).

In a recent paper (Chapter 3), it was shown that the pupal and adult weight were directly related to the duration of the latent feeding period in *C. jactatana*. On an artificial diet, final instar larvae that had longer latent feeding periods, had higher L_{MW}, pupal and adult weights. It was shown that the decrease in weight from L_{MW} to pupa was proportionately constant (D_P=0.3 and 0.25 of the L_{MW} for

males and females respectively). The drop in wet weight from pupa
to adult was also found to be proportionately constant (0.5 and 0.4
of the pupal weight for males and females respectively). The total
drop from L_{MW} to adult was named constant D_A, which for *C. jactatana*
was 0.6 and 0.5 of the L_{MW} for males and females respectively. As D_A
was constant over three generations of laboratory rearing, it was
proposed that any increase in the level of L_{MW} would increase adult
weight and hence improve reproductive performance and overall adult
quality. Increase in the duration of the latent feeding period would
increase food ingestion and assimilation during the latent feeding
period and therefore the adult weight.

The purpose of this paper is to investigate the effect of
three constant temperatures on the L_{CW}, latent feeding period, the
L_{MW} and subsequently, on the fecundity of *C. jactatana*.

5.3 MATERIALS AND METHODS

Neonate larvae of *C. jactatana* were obtained from a
laboratory population maintained for 12 generations on a general
purpose diet (GPD, Singh, 1983) at $20 \pm 1°C$, $75 \pm 5\%$ RH, LD, 18:6
(see Appendix A.1 for management and Table A.4 for diet composition)
and reared at $25°$, $20°$ and $15 \pm 1°C$, $75 \pm 5\%$RH, LD 18:6. To
investigate the effect of temperature on the L_{CW}, fifth instar larvae
(L_5) of medium size (head capsule width 1.18-1.20mm) were

transferred to plastic vials (40 X 100mm) after feeding for 12-
36hr after moult into L_5 (day 0), and starved for 20 days at
respective temperatures (n=30 for each temperature). Pupae formed
were sexed and weighed using a Shimadzu electronic balance (AEL-160)
with an accuracy of ±0.1mg. Larvae which had not began to pupate at
the end of 20 days were transferred to rearing test tubes containing
GPD diet and reared to pupation whereupon their sex was determined.

To investigate the effect of temperature on the latent
feeding period, L_5 larvae of medium size from the laboratory
population were transferred to plastic vials containing 1.5g of GPD
diet on day 0. Larvae were kept at the three constant temperatures
(n=60 at 25°, 20° and 15°C) and weighed individually at 24hr
intervals until pupation. Pupae were sexed, weighed on day of
pupation and incubated at 20 ±1°C until eclosion. Adults were
weighed and females (n=20) mated at 15 ±1°C, 75 ±5% RH, LD of 16:8,
with average sized males (about 30mg) from the laboratory
population. Data on female longevity, pre-oviposition and
oviposition period, and fecundity were obtained.

5.4 RESULTS

The L_{CW} for male larvae of 1.18mm and female larvae of 1.20mm
head capsule width, reared and starved at the three constant
temperatures were 28.7 ±0.7mg, 30.1 ±0.4mg and 29.3 ±0.9mg for males

and 35.6 ±1.1mg, 35.0 ±0.7mg and 35.8 ±0.6mg for females at 25°, 20° and 15°C respectively (all ± = 95% confidence intervals). The L_{CW} was not significantly different at the three temperatures.

Table 5.1 shows the results of the effect of temperature on the final instar of *C. jactatana*. The Pre-L_{CW} period decreased as temperature rose from 15° to 25°C (r= -0.9). The latent feeding period varied inversely with temperature (r=-0.92 for males and -0.98 for females). Temperature was inversely related with L_{MW} (r = -0.99 and -0.97 for males and females respectively). The linear regression equations are y=53.5-0.62x and y=68.4-0.86x for males and females respectively; where y is the L_{MW} and x the temperature. The equations explain for over 94% of the variation in L_{MW} (R^2= 97.8% for males and 94.5% for females) and are highly significant for the temperatures tested (p<0.01). For each 1° C increase in temperature there was about 1mg decrease in L_{MW}. L_{MW} was 32.1%, 40% and 53.4% of the L_{CW} for males and 32.0%, 38.9% and 55.8% of the L_{CW} for females at 25°, 20° and 15°C respectively. Pupal weights were also inversely related to temperature (r=-0.98). The decrease in wet weight from L_{MW} to pupa (D_P) was 30.7 ±1.9% and 26.3 ±2.4% at 25°C; 29.3 ±2.6 and 25.4 ±3.4 at 20°C; 27.1 ±3.1% and 26.8 ±4.5% at 15°C for males and females respectively and were not significant. Adult weights averaged 51 ±3% and 35 ±4.1% of the pupal weight for males and females respectively and were not significant (p>0.01).

Female longevity averaged 25.5 ±0.9 days and was not significant (P>0.01) for the three temperatures. Pre-oviposition and oviposition periods averaged 6.3 ±0.7 and 16.8 ±1.2 days respectively and were also not significant. Mean fecundity was 30 ±25; 137 ±31 and 194 ±26 eggs/female for females at 25°, 20° and 15°C respectively and was found to be significant (P<0.01 F=13, DF=29).

5.5 DISCUSSION

5.5.1 Effect of temperature on the L_{CW}

The results of the experiments show that L_{CW} is not affected by temperature and further supports the L_{CW}-mechanical stretch hypothesis (Nijhout, 1981). If the ultimate trigger of L_{CW} in initiating pupation is stretch related, then temperature during the pre-L_{CW} period would have no effect on the magnitude of L_{CW} (it has been hitherto shown that several generations of rearing and nutrition have no effect on the L_{CW}, see chapters 3 and 4).

Ratte (1985) proposed a dual temperature hypothesis to explain why insects are capable of accelerating or retarding their development under different temperatures. Ratte proposed that development was not achieved only by temperature compensation of the growth rate, but by modification of the critical size too. The L_{CW} in *C. jactatana* is fixed irrespective of difference within temperature range tested. The dual temperature hypothesis therefore

is likely more useful in explaining interspecies rather than intraspecies adaptation to temperature.

5.5.2 Effect of temperature on the latent feeding period

The choice of the three temperatures used in the experiment was based on earlier trials of total larval development at constant temperatures ranging from 10-25°C. A range of 15-25°C was found to be suitable for the rearing of *C. jactatana*. As L_{CW} is dependent on size (Jones, *et al.* 1981 and chapter 3), L_{CW} of the smallest male and female larvae were used in analysing the data (29mg and 36mg for males and females respectively). Errors introduced by this estimation are minimal as the difference between the L_{CW} of the largest and the smallest larva is less than 10mg, an amount a larva can ingest within a day.

The latent feeding period has been previously proposed as an invariable stage between two hormones (the initiation of decay of the juvenile hormone after attaining L_{CW} and the onset of the secretion of prothoracicotropic hormone, PTTH; see chapter 3 and 4). The length of the latent feeding period is in effect the period the juvenile hormone takes to decay (Bollenbacher and Granger,1985, Gilbert *et al.* 1980). Temperature effects are probably more complex affecting rate of juvenile hormonal secretion and catabolism. In *C. jactatana*, an increase in temperature may enhances juvenile hormone catabolism and hence shortens the duration of the latent feeding

period. Lower temperatures may decrease the catabolic efficiency of juvenile hormone esterases and hence prolong the latent feeding period. This hypothesis is consistent with that of Sweeney and Vannote (1981) who produced empirical evidence to explain the interaction of temperature, developmental processes and physiology in the determination of size and fecundity of six coexisting *Ephemerella* mayflies.

5.5.3 Effect of temperature on the L_{MW}.

Hegazi and Schopf (1984) using nutritional indices, showed that the physiological capacity of final instar *Spodoptera littoralis* (Boisd.) to consume and utilize food could be described in three reaction steps: feeding activity ---» digestion ---» efficiency to assimilate the digested food. At different temperatures, different sequences of steps limit the total reaction. Low feeding and assimilation affect the reaction at low temperatures while low digestion efficiency affect the reaction at high temperatures. Compensatory mechanisms seem to exist in the reaction to counter effect of temperature. There is high efficiency in digestion at low temperatures to compensate for the low assimilation, while high assimilation compensates reduced digestion at high temperatures. They further showed that optimum temperatures were coincident with high feeding, high digestion and low assimilation. In *C. jactatana*, the increase in latent period of feeding due to temperature decrease probably increases total digestion and assimilation time, promoting

growth and hence allows for more feeding. The higher L_{MW} level
recorded at 15°C is therefore likely due to increased total food
intake during the latent feeding period.

Manduca sexta final larval instar show higher total food
consumption at 20°C but decrease progressively with increasing
temperatures to 35°C (Reynolds and Nottingham, 1985).
Absorption of food from the gut was constant over the temperature
range. Although larvae had an increased rate of growth at higher
temperatures, their final size was higher at lower temperatures. The
inverse relation between final size and temperature in *M. sexta* could
not be adequately explained by Reynolds and Nottingham. It is likely
that in *M. sexta*, the inverse relation between size and temperature
is dependent on the effect of temperature on the duration of the
latent feeding period. It is possible, for example, that the
activation of the prothoracic gland and release of PTTH may occur at
a slower rate at lower temperatures and hence a prolonged latent
feeding period.

5.5.4 Temperature during the final instar and adult reproductive
performance

The result of the present study corroborates the earlier
report of Danthanarayana (1975) who recorded cooler temperatures
producing heavier females with increased longevity, extended
oviposition periods and higher fecundity in the leafroller, *Epiphyas*

postivittana (Walker). In nature, heavier and more fecund females were hence more frequent in winter and the lighter individuals common in summer. Since his laboratory results under similar temperatures were consistent with the field results, it is possible that in *E. postivittana*, temperature has a similar effect as in *C. jactatana* during the final larval instar. Similarly, in the tobacco budworm, *Heliothis virescens*, Nadgauda and Pitre (1983) found females were more fecund at 20° than at 25° and 30°C.

Figure 5.1(a-g) shows scatterplots of pupal weights of *C. jactatana* reared under 7 constant temperatures. Pupal critical weights (P_{CW}) for males and females plotted within the scatterplots are derived from the L_{CW} and D_P (see chapter 3):-

$$P_{CW} = L_{CW} - (L_{CW} \times D_P)$$
$$P_{CW} \, \male = 29 - (29 \times 0.3) = 20.3 mg$$
$$P_{CW} \, \female = 36 - (36 \times 0.25) = 27.0 mg$$

Overall, the pupal weights do not fluctuate greatly. Pupal weights for both sexes are heavier than the P_{CW} weight. As temperatures approach extremes (10° and 25°C), pupal weights tend to approach the P_{CW}. A temperature range of 12-18°C spreads pupal weights away from the critical limits. Although a temperature range of 10-25°C supports development, reproduction is hampered at the extremes and is optimum at a temperature range of 12-15°C. Sweeney

and Vannote (1978) define an optimal larval temperature as one that permits an insect to achieve maximum adult weight and fecundity (note also Wright and Mattice, 1981). In relation to P_{CW} in C. *jactatana*, optimum temperatures produce the widest shift of pupal and adult weights above the critical limits leading to increased fecundity. Optimum temperatures produce pupae and adults of high quality index value (Chapter 3) compared to sub-optimal temperatures.

The smaller body weight resulting from extreme temperature is overall costly to the insect as it results in low offspring production. Reproductive performance is optimal where size is greatest. Overall adult quality is also likely linked to increase in weight (see Smith, 1986,). Though the developmental rate of insects reared at constant conditions is not truly reflective of natural field conditions (Ratte, 1985), the results exhibit a consistency with field data of similar holometabolous insects.

5.6 CONCLUSION

The results of this investigation indicate that L_{CW} is temperature independent in $C. jactatana$. Lowering the developmental temperature from 25°C to 15°C increases both the duration of the latent feeding period and the L_{MW} and therefore leads to heavier pupae and adults which are more fecund. Increase in latent feeding

period due to lowering of temperature is likely to increase the overall quality of adults. The effect of temperature in regulating the L_{MW} and not the L_{CW} is at variance with Ratte's dual temperature hypothesis but supports the L_{CW} mechanical-stretch hypothesis. The actual effect of temperature is probably mediated through the neuro-endocrine system (by delaying the decay of the juvenile hormone or the secretion of PTTH).

Table 5.1: Effect of temperature on the final

instar of *C. jactatana*

* at $75 \pm 5\%$RH

** mean «$\pm$»95% confidence intervals

Rows followed by the same letter are not

significantly different ($p > 0.01$)

		Constant temperature[*]		
		25 ±1°C	20 ±1°C	15 ±1°C
n	♂	41	39	42
	♀	17	20	18
Duration of L_5 (days)	♂	10.6a[**] 10.3«±»10.9	12.7b 12.1«±»13.3	30.4c 29.3«±»31.5
	♀	10.5a 10.1«±»10.9	13.4b 13.0«±»13.8	32.4c 30.5«±»34.3
Pre-L_{OW} period (days)	♂	1.8a 1.5«±»2.1	2.0a 1.9«±»2.1	6.4b 4.9«±»7.9
	♀	1.5a 1.1«±»1.9	2.1a 1.7«±»2.5	5.8b 4.2«±»7.4
Pre-L_{MW} period (days)	♂	4.3a 4.2«±»4.4	5.1a 4.3«±»5.9	14.5b 12.8«±»16.1
	♀	4.4a 4.1«±»4.7	6.3b 5.8«±»6.8	14.9c 12.5«±»17.3
Pre-pupal period (days)	♂	5.1a 4.8«±»5.4	6.6b 6.0«±»7.2	16.2c 14.7«±»17.7
	♀	5.1a 4.7«±»5.5	6.0a 5.5«±»6.5	17.7b 15.0«±»20.4
Latent feeding period (days)	♂	2.7a 2.4«±»3.0	3.4a 2.8«±»4.0	8.3b 7.2«±»9.4
	♀	2.9a 2.5«±»3.3	4.8b 4.3«±»5.3	9.2c 7.4«±»11.0
L_{MW} (mg)	♂	38.3a 36.1«±»40.5	40.6a 38.1«±»43.1	44.5b 43.2«±»45.8
	♀	47.5a 45.3«±»49.7	50.0a 48.3«±»51.7	56.1b 54.4«±»57.8
Pupal weight (mg)	♂	26.1a 25.2«±»27.0	28.1a 26.2«±»30.0	31.5a 28.2«±»34.8
	♀	35.6a 33.7«±»37.5	37.0a 35.7«±»38.3	39.7a 37.6«±»41.8

Figure 5.1: Scatterplots of pupal weights of

C. *jactatana* reared under 7 constant

temperatures

a) 10°C

(n=56 for male

41 for female)

Figure showing a scatter plot of Pupal weights (mg) on the x-axis ranging from 18 to 58.5, with Male pupal weights (*) and Female pupal weights (✿) plotted. Vertical lines indicate Male P_{cw} (near 21) and Female P_{cw} (near 27).

Figure 5.1: Scatterplots of pupal weights of

C. *jactatana* reared under 7 constant

temperatures

b) 12°C

(n=64 for male

63 for female)

Male P_{cw}

Female P_{cw}

* Male pupal weights
☆ Female pupal weights

Pupal weights (mg)

18   22.5   27   31.5   36   40.5   45   49.5   54   58.5

Figure 5.1: Scatterplots of pupal weights of

C. *jactatana* reared under 7 constant

temperatures

c) 15°C

(n=44 for male

39 for female)

Male P_cw   Female P_cw

* Male pupal weights
✡ Female pupal weights

Pupal weights (mg)

18   22.5   27   31.5   36   40.5   45   49.5   54   58.5

Figure 5.1: Scatterplots of pupal weights of

C. *jactatana* reared under 7 constant

temperatures

d) 18°C

(n=49 for male

48 for female)

Male P$_{cw}$ Female P$_{cw}$

* Male pupal weights
✡ Female pupal weights

18   22.5   27   31.5   36   40.5   45   49.5   54   58.5

Pupal weights (mg)

Figure 5.1: Scatterplots of pupal weights of

C. *jactatana* reared under 7 constant

temperatures

e) 20°C

(n=50 for male

53 for female)
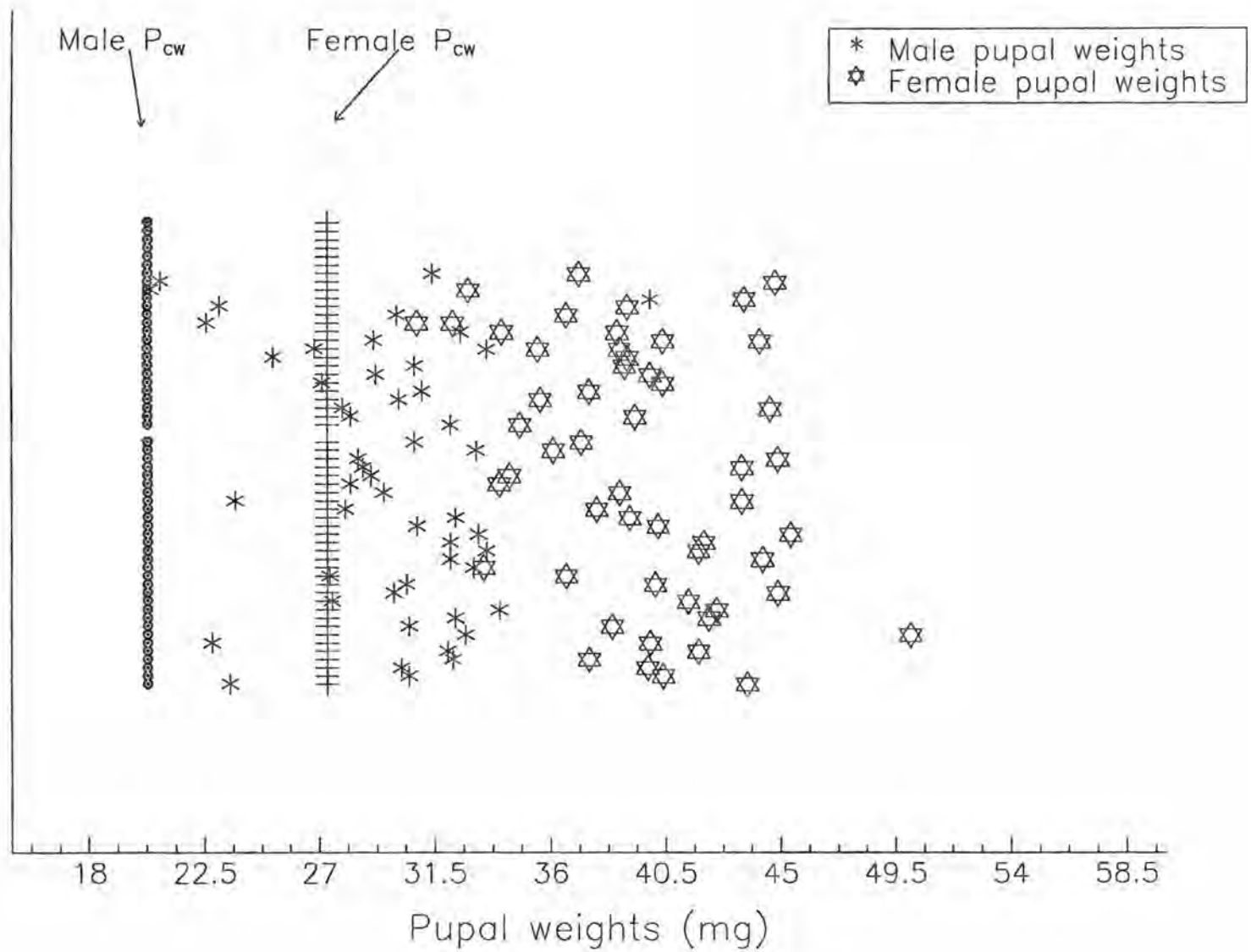
Figure 5.1: Scatterplots of pupal weights of

C. *jactatana* reared under 7 constant
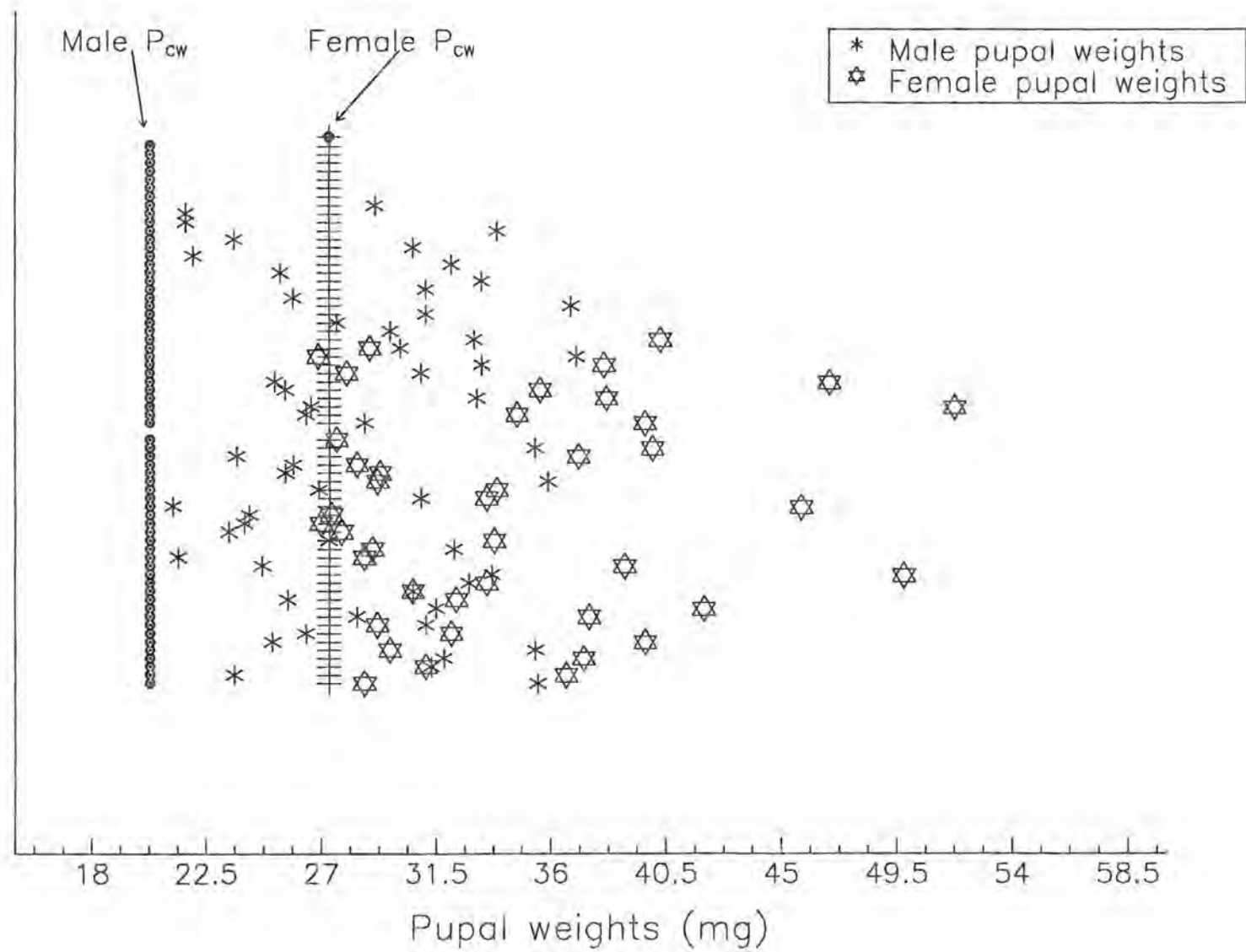
temperatures

f) 22°C

(n=59 for male

57 for female)
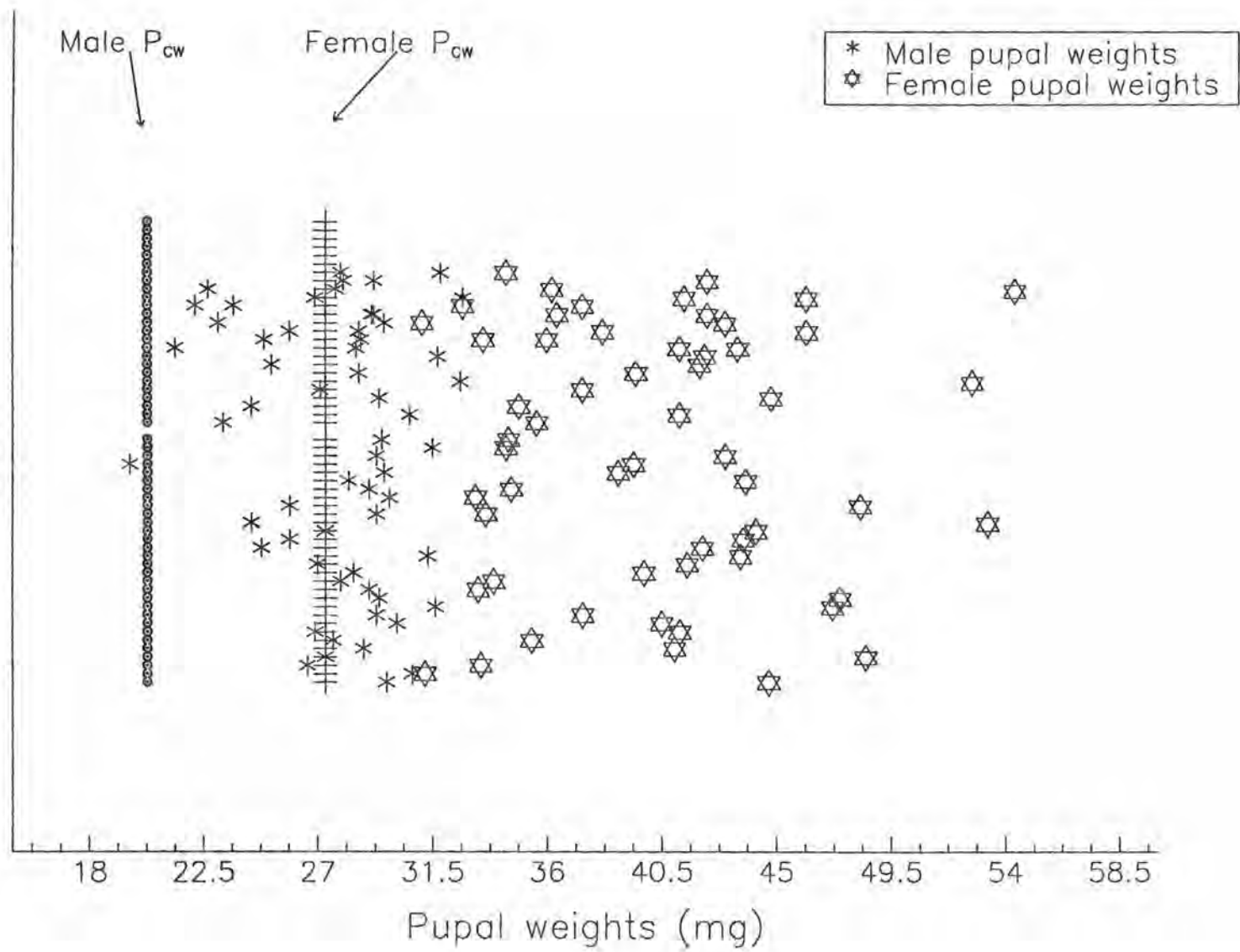
Figure 5.1: Scatterplots of pupal weights of

C. *jactatana* reared under 7 constant

temperatures
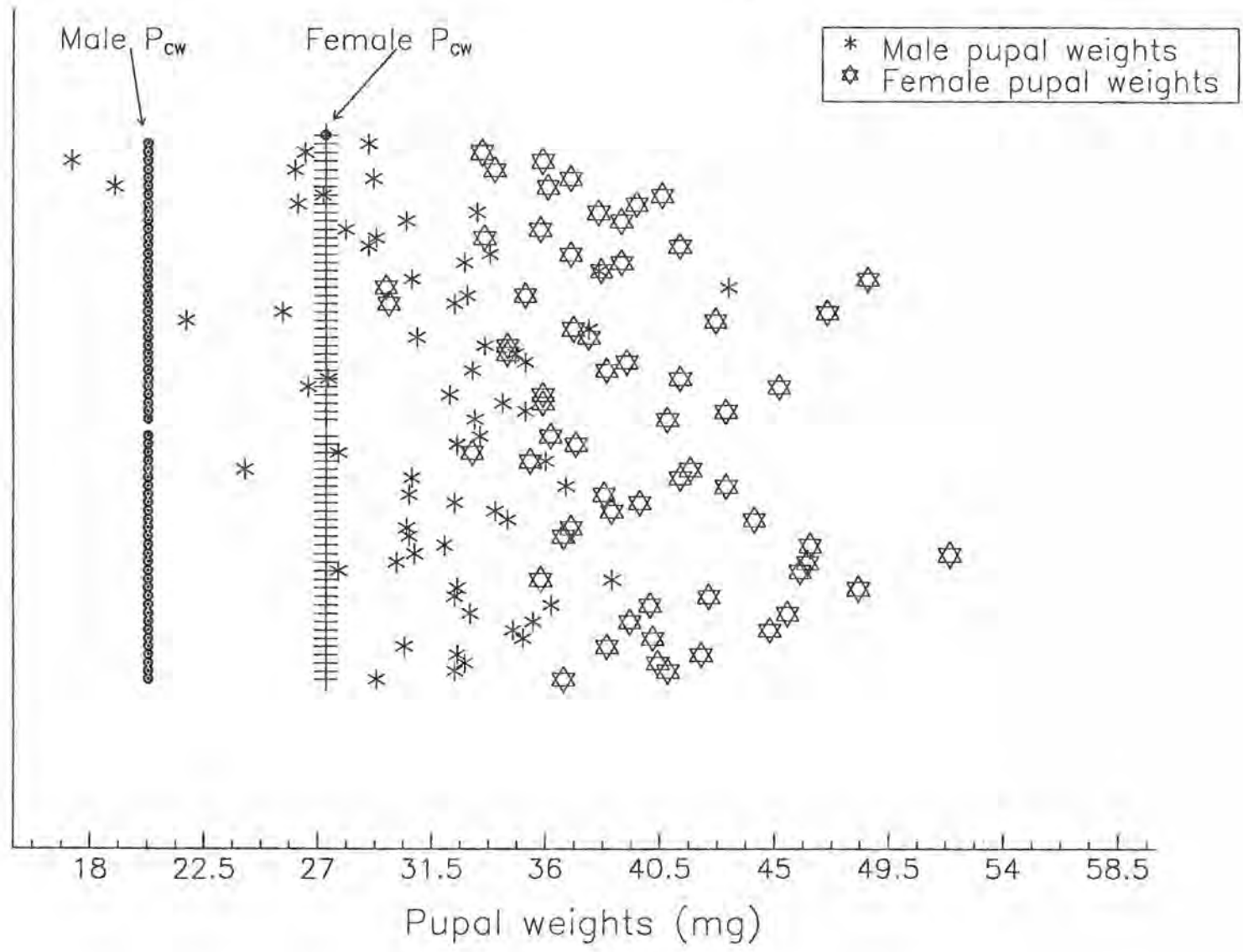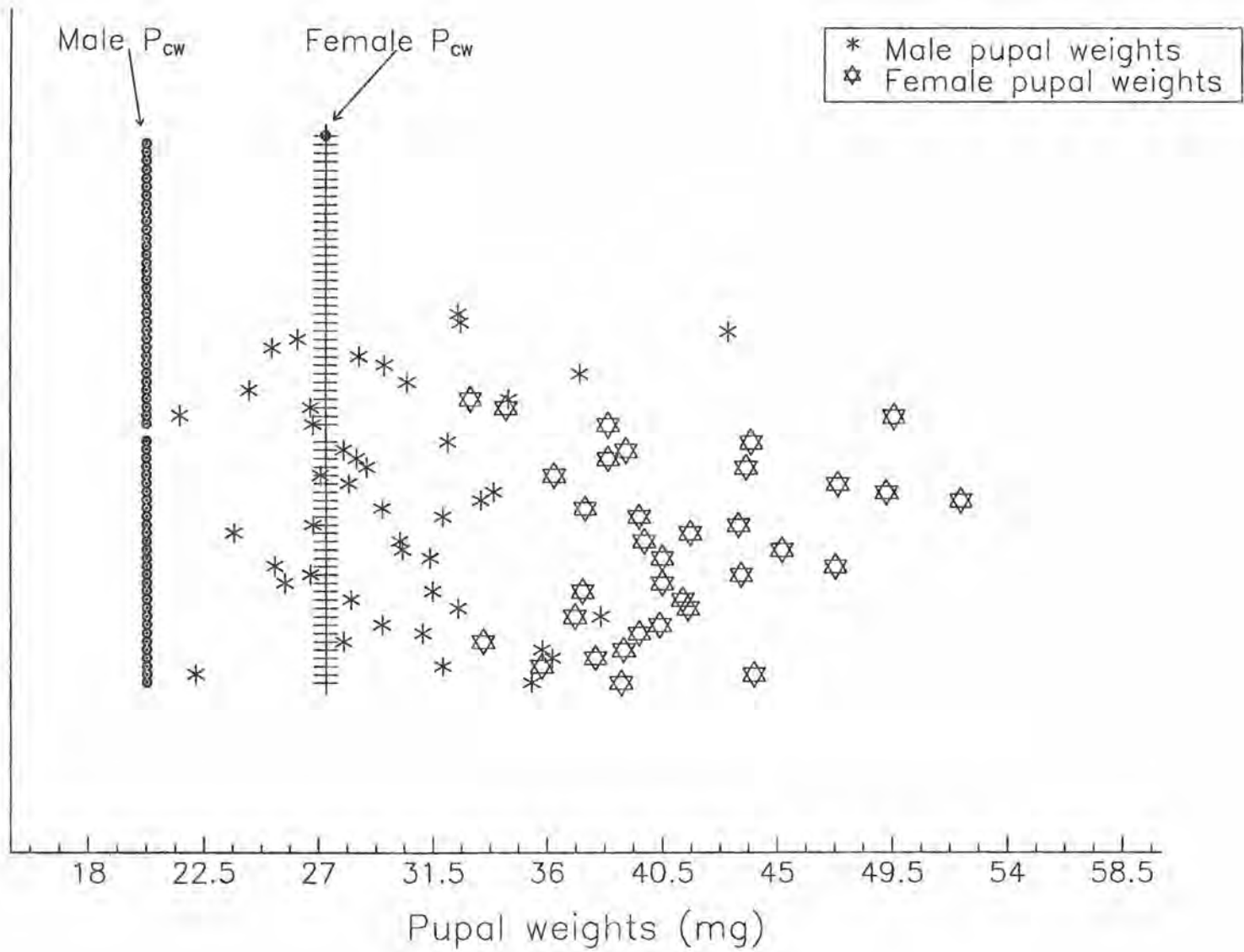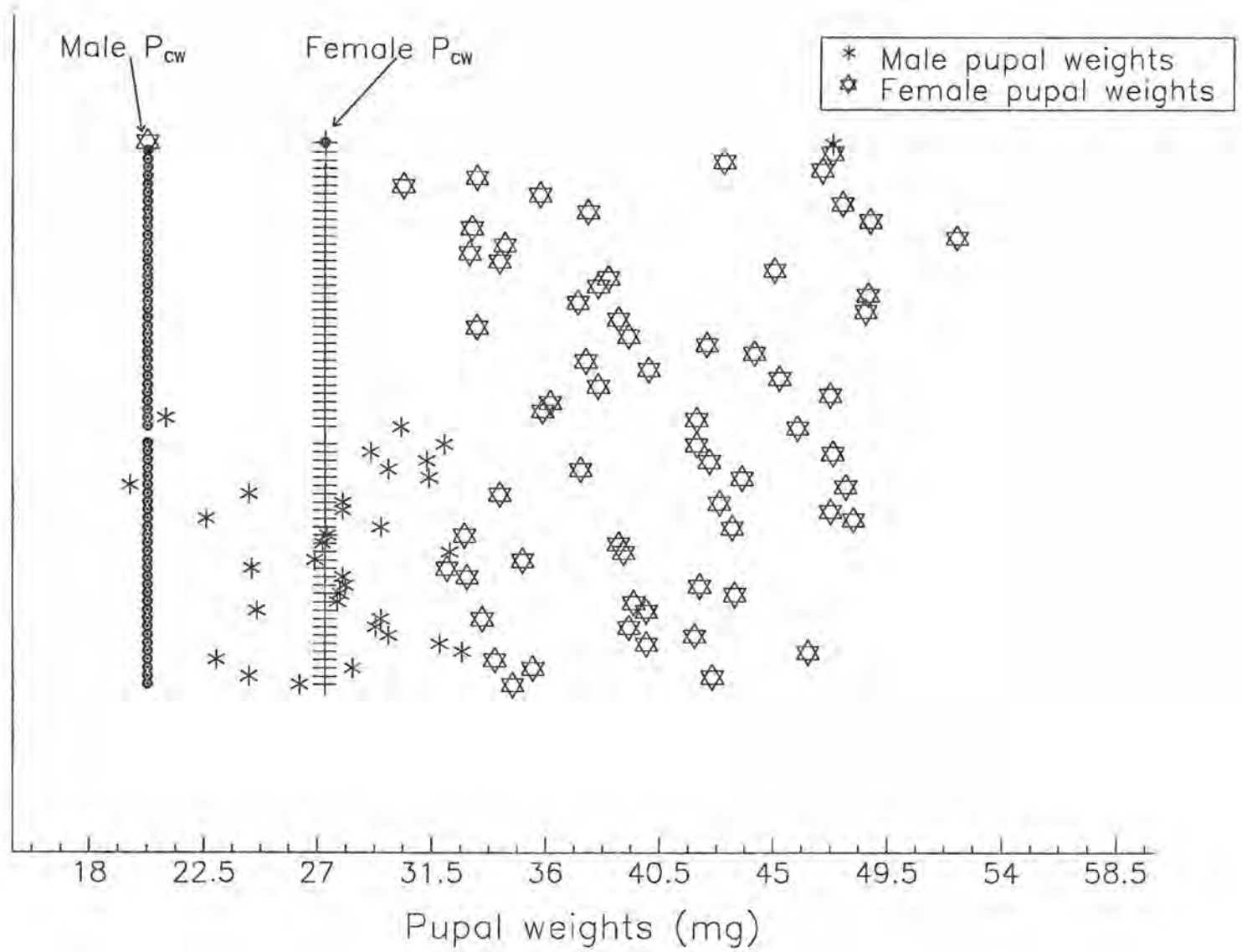
g) 25°C

(n=47 for male

47 for female)
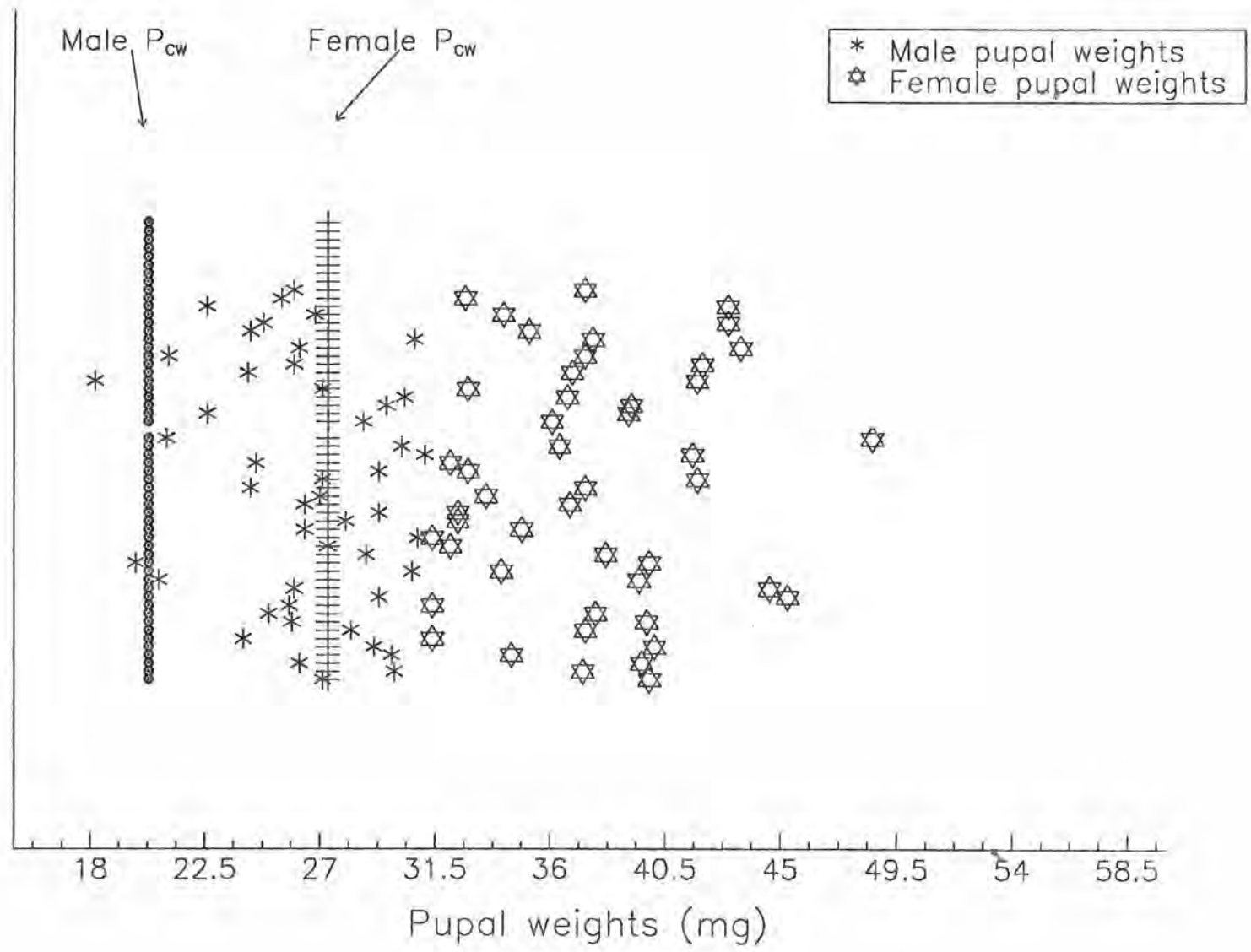
Male P_cw   Female P_cw

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 *  Male pupal weights
 ✿  Female pupal weights
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Pupal weights (mg)

18   22.5   27   31.5   36   40.5   45   49.5   54   58.5

CHAPTER / 6

THE EFFECT OF THREE ABIOTIC FACTORS ON THE LARVAL CRITICAL WEIGHT,
LATENT FEEDING PERIOD, LARVAL MAXIMUM WEIGHT AND FECUNDITY OF
'*Cnephasia*' *jactatana* (Walker) (Lepidoptera: Tortricidae)
II. PHOTOPERIOD & III. THERMOPHOTOPERIOD

6.1 ABSTRACT

The starving of final instar larvae (L_5) of '*Cnephasia*'
jactatana under five photoperiodic regimen (LD 24:0, 18:6, 12:12,
6:18 and 0:24) had no effect on the larval critical weight (L_{CW}).
Photoperiod, however, had significant effects on the duration of L_5.
Photoperiods of LD 24:0, 18:6 and 0:24 prolonged L_5 more than those
of LD 6:18 and 12:12. Generally, an increase in the photophase
decreased the duration of L_5. Larval maximum weight (L_{MW})
correlated positively with the latent feeding period for both males
and females. Reproductive performance was not significant for
females reared under the five photoperiods. A thermoperiod of 14-
22°C interacted significantly with a photoperiod of LD 12:12. Longer
latent feeding periods, higher L_{MW}, pupal weights and fecundity
occurred where the thermophase coincided with the photophase. These
results are related likely to the gated release of the
prothoracicotropic hormone (PTTH). The results also suggest that

photoperiods and thermophotoperiods should be considered in discussions relating to the biological performance and overall quality of insect species.

6.2 INTRODUCTION

Various developmental process in insects are under the external control of photoperiod and temperature (Danilevsky, 1965; Beck, 1980; 1983b; Saunders, 1982; Denlinger, 1985; Ratte, 1985). Processes that occur only once in the life cycle of an insect (e.g. egg hatch, final larval ecdysis, pupation, eclosion, diapause) are often under circadian control and are 'gated' (Zdàrek, 1985; Truman, 1985; Page, 1985). The pre-pupal phase of many Lepidoptera begins with the gated release of the prothoracicotropic hormone (PTTH, Truman, 1972; Truman and Riddiford, 1974; Safranek and Williams, 1980; Nijhout, 1981; Jones *et al.* 1981; Fujishita and Ishizaki, 1981). A second release of PTTH has been shown to occur in the final instar of *Manduca sexta* and *Trichoplusia ni* which is not photosensitive (Truman and Riddiford, 1974; Jones *et al.* 1981; Bollenbacher and Granger, 1985). The 'gated' release of the PTTH is thought to be temperature compensated (Truman, 1972; Page, 1985).

In the fifth and final larval instar (L_5) of the black lyre leafroller, *'Cnephasia' jactatana* (Walker), pupation is initiated after attaining a larval critical weight (L_{CW}=29mg and 36mg for L_5

larvae of head capsule width of 1.18 and 1.20mm in males and females respectively; see chapter 3). The attainment of an L_{CW} in *M. sexta* (Nijhout and Williams, 1974a) and in *T. ni* (Jones *et al.* 1981) has been demonstrated to initiate steps that ultimately stop the secretion of the juvenile hormone. During the period that the juvenile hormone takes to decay from the haemolymph, the larvae continue feeding (latent feeding period), attaining weights above the L_{CW}. The highest wet weight attained in L_5 of *C. jactatana* at 20°C is after 4-6 days after moult and is referred to as the larval maximum weight (L_{MW}, see chapter 3, Figure 3.4) and is thought to coincide with the release of PTTH.

Several workers have reported that fluctuating temperatures have developmental effects on insects (e.g Messenger, 1964; Hagstrum and Hagstrum, 1970; Hagstrum and Leach, 1973; Beck 1983a). Fluctuating temperatures can have marked effects on the adult size and weight; causing larger adults in the pink bollworm *Pectinophora gossypiella* (Welbers, 1975) and smaller adults in *Spodoptera littoralis* (Sidibè and Laugè, 1977; see also Ratte, 1985 for detailed review). Beck (1985a, 1987) introduced the term 'thermophotoperiod' to define the superimposition of thermoperiod on photoperiod.

This paper investigate the influence of photoperiod and thermophotoperiod on the L_{CW}, latent feeding period, L_{MW} and

reproductive performance of *C. jactatana.*

6.3 MATERIALS AND METHODS

Hatched larvae were obtained from a laboratory population that had been maintained for 12 successive generations on a general purpose diet (GPD, Singh, 1983; see Table A.4 for composition and Appendix A.1 for details of the rearing technique).

To determine whether photoperiod had any effect on the L_{CW}, larvae were reared from neonate until molt into L_5 (day 0) in 24 litre light controlled micro-cabinets (40 X 30 X 30cm with a 15W tungsten filament lamp; Muggleston 1986) set at LD 24:0, 18:6, 12:12, 6:18 and 0:24 and at 18 $\pm$1°C, 75 $\pm$5%RH. Lux meter recordings gave a reading of 0.03 lux in darkness and 770 lux when the lamp was on. L_5 larvae of medium size (head capsule width of 1.18-1.20mm) were allowed to feed for 12-24 hrs before transfer to plastic vials (40 X 100mm) and starved at respective photoperiodic regimen of rearing for 20 days (n=30 for each regimen). Larvae not pupated by the end of 20 days were transferred to rearing test tubes containing GPD diet and reared until pupation whereupon their was sex determined.

To determine the effect of photoperiod on the latent feeding period and L_{MW}, L_5 larvae of medium size reared from neonate stage at

18± 1°C, 75 ±5%RH, LD 0:24; were transferred to plastic vials containing 1.5g of GPD diet and reared until pupation in the micro cabinets set at the 5 photoperiodic regimens (n=60 for each regimen).

To determine the effect of thermophotoperiods, L_5 larvae of medium size (also reared from neonates at LD 24:0 and 20 ±1°C, 75 ±5%RH) were reared from day 0 in a Precision Scientific incubator set at LD 12:12, 22-14°C. In the first experiment, the warm phase (thermophase) coincided with the photophase while in the second experiment, the thermophase coincided with the scotophase (n=112 and 120 respectively).

All larvae (except those starved and those reared at LD 0:24) were individually weighed every 24 hrs during the photophase using a Shimadzu electronic balance (AEL-160) with an accuracy of ±0.1mg. Larvae under 24hr darkness were weighed in dark with the use of a flashlight covered in a red filter and a layer of tissue paper. Pupae were sexed, weighed and incubated until eclosion. Adults eclosing were also weighed before mating the females (n=20) at 15 ±1°C, 75 ±5%RH, LD 16:8 with average sized males (about 30mg) from the laboratory population and results of female fecundity obtained. Each experiment was repeated.

Statistical analyses were carried out by use of the

computerized statistical system known as Minitab (Ryan *et al.* 1985).

6.4 RESULTS

The L_{CW} for L_5 starved at the 5 photoperiods did not vary and averaged 29.6 $\pm$0.6mg and 35.5 $\pm$1.0mg for male and female larvae of 1.18mm and 1.20mm head capsule width respectively (all $\pm$ = 95% confidence intervals).

Data on the effect of photoperiod on the L_5 of *C. jactatana* are presented in table 6.1. In analysing the data, L_{CW} was taken to be 29.0mg and 36.0mg for male and female larvae respectively. Total duration of L_5 (for both male and female) was significantly different (F=17.3, DF=77, p<0.01). LD 12:12 and 6:18 had the shortest durations, while there was a progressive increase from about 13 to 15 days for LD 24:0, 18:0 and 0:24 (see Figure 6.1). L_5 durations were not significantly different (p> 0.05) within the short L_5 duration group (LD 12:12 and   6:18) but were significant (p<0.01) from the long L_5 duration group (24:0, 18:0 and 0:24). Within the long L_5 duration group, LD 0:24 was significantly longer than 24:0 (p< 0.01). A correlation of the duration of photophase and duration of L_5 was not significant when considering all the treatments (r=-0.15) but highly significant considering only the long L_5 duration group (r=-0.99). A similar pattern was obtained

for male and female L_5 durations (r=-0.07 and -0.27 for all treatments and r=-0.97 and -0.99 in the long L_5 duration group for males and females respectively). In other words, with an exception of the short duration group, as the photophase decreased from 24 to 0hrs there was an increase in total time spent at L_5.

Correlation of the latent feeding period and length of the photophase was not significant (r=+0.22 and +0.53 for males and females). Latent feeding periods fell into two significant categories (p<0.05), low latent feeding periods for LD 12:12 and 6:18 and high latent feeding periods for LD 24:0, 18:0 and 0:24. Correlation of the duration of the photophase was not significant for males in the latter category (r=+0.24) but was highly significant for females (r=1.0). Pre-pupal periods were not significant at any level.

The larval maximum weight and the photophase gave a correlation coefficient of +0.37 for both males and females. In both sexes, the L_{MW} was high for LD 0:24 and low for LD 6:18. Correlation between L_{MW} and the latent feeding period was significant at r=+0.91 for males and +0.57 for females. Pupal weights were not significantly different at any level (P>0.05). The decrease in weight from L_{MW} to pupal weight (D_P) was not significant and averaged 31.1 ±1.6% and 24.7 ±1% for males and females respectively. The total decrease in weight from L_{MW} to adult (D_A) was also not

influenced by photoperiod averaging 61.4 ±3.2% and 50.5 ±9.9% for males and females respectively.

Table 6.2 shows the results of the effect of thermophotoperiod on the final instar of *C. jactatana*. The duration of L_5, the latent feeding periods, L_{MW}, and pupal weights were significant in the two treatments for both sexes ($p<0.01$). Pre-pupal periods were not significant at any level ($P>0.05$). In the treatment where the thermophase coincided with the photophase, there was significantly longer latent feeding periods, higher L_{MW} and pupal weights than where the thermophase coincided with the scotophase ($p<0.001$). In comparing thermophotoperiod and constant temperature of rearing, it was found that where the thermophase coincided with the photophase was not significantly different from LD 12:12 at 18°C. Constant temperature rearing had significantly longer latent feeding periods, L_{MW} and pupal weights than the thermophotoperiod that coincided thermophase with scotophase. D_P and D_A were not affected by phase relation and were not different from those from the five photoperiods.

Fecundity of females reared under the different photoperiods was variable averaging 157 ±32 eggs/female and was not significant. The fecundity of females reared under the thermophotoperiods was 180 ±41 eggs/female for the thermophase with photophase and 102 ±39 eggs/female for the thermophase with scotophase. Fecundity was

significantly different at p<0.05.

Synchronism indices (relative number of male and female coincidences in pupation (S_P) and eclosion (S_A), see chapter 2 for details) showed no distinct patterns. S_P were lowest under LD 0:24, 18:6 and 6:18 (0.4) and highest under 24:0 (0.7). S_P was high (0.8) in the thermophotoperiod where thermophase coincided with photophase compared to where the thermophase coincided with scotophase (0.6).

6.5 DISCUSSION

6.5.1 Photoperiod and the L_{CW}

Since the results indicate that the L_{CW} is not influenced by photoperiod, supports the mechanical-stretch hypothesis of a critical weight (Nijhout, 1981). An L_{CW} determined by mechanical means would unlikely be under the influence of photoperiod. It has earlier been demonstrated that the L_{CW} for *C. jactatana* is not affected by successive generations of laboratory rearing, diet or temperature (see chapters 2, 3 and 4). This finding is at variance with Palmer (1982) who observed a downward shift in the L_{CW} of final instar larvae of the milkweed leaf beetle, *Labidomera clivicillis* reared under short photoperiods. Palmer does not mention the size of the larvae he used for his starvation experiments. It is possible, therefore, that the shorter photoperiod had decreased the size of

earlier instars leading to smaller final instar larvae. Since L_{CW} is size dependent (see chapter 3, Nijhout, 1981 and Jones *et al.* 1981), smaller larvae would have lower L_{CW} and pupate at lower weights.

6.5.2 Photoperiod, latent feeding period and the L_{MW}

The role of photoperiod in the L_5 of *C. jactatana* appears complex. Generally, as the photophase decreased, the larvae spent longer at L_5, had longer latent feeding periods and higher L_{MW}. Since the Pre-L_{CW} periods were not significantly different in any of the treatments, and since L_{CW} was not influenced by photoperiod, it appears that the the effect of photoperiod is related to events during the latent feeding period. Attainment of L_{CW} triggers events that lead to the cessation of the secretion of the juvenile hormone by the corpora allata. The presence of the juvenile hormone prevents the secretion of PTTH by the brain and must therefore be cleared from the haemolymph before the brain is able to release PTTH. During the process of clearing the juvenile hormone, the larvae continue to feed. The latent feeding period is the time spent from attaining L_{CW} until the secretion of PTTH (Nijhout and Williams, 1974a). Nijhout and Williams (1974b) showed that in *Manduca sexta*, PTTH release was 'gated' and dependent on photoperiod. In experiments with *C. jactatana* the day the highest wet weight was attained by an L_5 larva (L_{MW}) is taken to be the day of PTTH release.

Two models can be used to explain the role of photoperiod in the final instar of *C. jactatana*. Beck (1985b) postulated the existence of a dual system of biological clocks (DST). The S-system constitutes the photoperiodically entrained circadian pacemaker. The P-system serves as a gating rythmn. The S-system is entrained by photoperiod and is free-running under continuous darkness (LD 0:24) but it stops under continuous light (LD 24:0) or long photoperiods. The P-system is entrained by the S-system during scotophase and continuous darkness, but is free-running in photophase and continuous light. In *C. jactatana*, LD 0:24 has longer L_5 duration because the S-system is free-running and therefore cannot entrain the P-system. Release of PTTH is delayed leading to longer latent feeding periods, increased feeding and high levels of L_{MW}. In LD 24:0, the S-system stops and cannot entrain the P-system which then free-runs and release of PTTH is also delayed. It appears that LD 18:6 is probably a long photoperiod that stops the S-system or a short scotophase not enough to desaturate the P-system and hence no proper phase relationship is achieved by the time the larvae are able to secrete PTTH leading to a delay in the release of the hormone. LD 12:12 and 6:18 probably regulate the two systems quicker and hence PTTH release is not delayed resulting in shorter latent feeding periods.

The second model is the damped circadian oscillator of Lewis and Saunders, (1987). The circadian model includes a 'clock' and

'counter' mechanism. The oscillator involves a chemical concentration oscillating in relation to a reference value. Synthesis of the chemical is controlled through a delay system. Light breaks down the chemical, reducing its concentration. The photoinducible phase occurs when the concentration falls through a threshold value. When the threshold value is crossed in the dark a hypothetical active substance (named INDSUM) is produced. It is possible that in *C. jactatana* , LD 0:24 and 24:0 are free running, the oscillator damps out and little INDSUM is produced leading to a delayed release of PTTH. Long photoperiods result in less INDSUM than shorter photoperiods. PTTH release is therefore delayed in long photoperiods than in short photoperiods.

These findings are in agreement with Bogus' *et al.* (1987) who found that in *Galleria mellonella* reared under LD 12:12, final instar larvae terminated feeding and pupated 1 day earlier than larvae reared under 0:24. Bogus' *et al.* did not relate their findings to the gating rythmn of PTTH but rather to differential juvenile hormone and esterase titre. In *Drosophila melanogaster*, Roberts *et al.* (1987) showed that photoperiod influenced departure of final instars from food. Endo and Murakami (1985) demonstrated photoperiodic effect on morph size of the swallowtail butterfly, *Papilio xuthus* .

6.5.3 Thermophotoperiods, latent feeding period and the L_{MW}

In natural environments, temperatures fluctuate considerably, but usually in a regular daily pattern. Thermoperiodic responses are periodic and can mimic photoperiods (Saunders, 1973; 1982). Daily thermoperiods and photoperiods synchronise so that the thermophase coincide with the photoperiod and the cold phase (cryophase) coincide with the scotophase. Thermoperiod has been described as having a supplementing or modifying effect to photoperiod (Chippendale *et al.* 1976; Rock, 1983). Beck (1985a; 1987) demonstrated that the duration of cryophase together with scotophase had greater effect in the induction of diapause in *Ostrinia nubilalis* than combined thermophase and photophase. Allsopp (1986) found that models derived from constant-temperatures of rearing generally underestimated development. Thermal summation models based on fluctuating temperatures are better than models from constant temperatures, but still underestimate development considerably. 'Synergism' was a term used by Greg (1982) to describe the effect of unknown factors influencing development only under fluctuating regimens (see also Messenger and Flitters, 1959). Beck's review on insect thermoperiodism (1983b) noted that few studies had on dealt exhaustively with the effects of thermoperiods and thermophotoperiods on insect development.

Two explanations are possible for the effect of coinciding the thermophase with photophase on the development of L_5 of *C*.

jactatana; a periodic and non-periodic explanation. A periodic explanation would be that thermophase during scotophase serves as a powerful stimulus to synchronise and entrain the system that determines PTTH release. Synergism between feeding, photoperiod and thermoperiod might be a non-periodic explanation (see Beck, 1983a). It is possible that phase relationship between thermoperiod and photoperiod might stimulate neuroendocrine process differently. The magnitude of the difference between the latent feeding period of the two phases suggests that a warm phase coinciding with the photophase might either increase the secretion of the juvenile hormone or delay its catabolism.

6.5.4 Photoperiods, thermophotoperiods and reproductive performance

Fluctuating temperatures have been associated with faster development so long as the temperatures are within the optimal range of the species. Hagstrum and Leach (1973) showed that fluctuating temperatures caused an increase in the fecundity of some Coleoptera. In the black cutworm *Agrotis ipsilon*, larval growth is faster under long-day photoperiods (LD 16:8) than under short-day photoperiods (LD 12:12, 16:8) at 23°C. At lower temperatures (16-19°C), the converse was true, short-day photoperiods accelerated larval development. A thermophotoperiod of LD 12:12, 20-10°C stimulated greater weight gain than rearing under constant temperature (Beck, 1986). In caterpillars of *Dasychira pudibunda* (L.), short day (LD 10:14) with lower temperatures (15°C) increased the growth

rate and larval weight while long days (LD 24:0) with high temperatures (25°C) decreased the both the growth rate and larval weight (Geyspits and Zarankina, 1963).

In *C.jactatana* , photoperiod in the last instar affects the L_{MW} level and hence pupal and adult weights. The effect, however, is not of a magnitude so as to affect reproductive performance. More experimentation is needed to determine precisely the effect of photoperiod on overall quality. Phase relationship between photoperiod and thermoperiod has a pronounced effect on the level of L_{MW} and therefore pupal and adult size. A coincidence of the thermophase with the photophase produced females which were more fecund.

6.6 CONCLUSION

The results indicate that although L_{CW} is not effected by photoperiods, development of the fifth instar larvae of *C. jactatana* is under the control of photic and thermal conditions. It is suggested that thermophotoperiodic stimulation of feeding and growth is mediated through a neuro-endocrine sequence that probably affects the gated release of PTTH. Photic and thermal conditions have an effect on the adult reproductive performance and most likely on the overall quality. Increase in wet weight is consistent with findings of Beck (1982, 1983a, 1986) working on final instars *A. ipsilon* and

O. nubilalis. The results of the present experiment are not enough to determine what aspects of growth of the final instar of *C. jactatana* are under periodic effect. The adaptive significance of photoperiod and thermophotoperiod is not well documented, although longer photophase might lead to a shorter development time in the field (note Philogene, 1982). The results also indicate that thermophotoperiods might have metamorphic significance and should be included in models for insect development.

Table 6.1: Effect of photoperiod in the final

instar of *C. jactatana*

* at 18 ±1°C, 75 ±5%RH

** mean «±» 95% confidence intervals

Rows followed by the same letter are not

significantly different (p>0.01)

		Photoperiodic regimen*				
		LD 24:0	LD 18:6	LD 12:12	LD 6:18	LD 0:24
N	♂	34	30	22	36	26
	♀	26	30	33	24	34
Duration of L_s (days)	♂	13.1a 12.6«±»13.6**	13.7a 13.2«±»14.2	11.4b 10.9«±»11.9	11.7b 11.4«±»12.0	14.4a 12.8«±»16.0
	♀	13.5a 13.0«±»14.0	14.1a 12.8«±»15.4	12.5b 12.1«±»12.9	12.6a 11.7«±»13.5	15.1c 14.0«±»16.2
Pre-L_{ow} period (days)	♂	2.3a 2.0«±»2.6	2.4a 2.2«±»2.6	2.0a 1.7«±»2.3	2.1a 1.8«±»2.4	2.7a 2.2«±»3.2
	♀	2.4a 2.0«±»2.6	2.4a 2.1«±»2.7	2.2a 2.0«±»2.4	2.5a 2.1«±»2.9	3.0a 2.4«±»3.6
Pre-pupal period (days)	♂	6.0a 5.7«±»6.3	6.1a 5.6«±»6.6	5.8a 5.0«±»6.6	5.5a 5.2«±»5.8	6.3a 4.3«±»8.3
	♀	6.1a 5.8«±»6.4	6.9a 6.0«±»7.8	6.1a 5.3«±»6.9	6.0a 5.5«±»6.5	7.7a 6.5«±»8.9
Latent feeding period (days)	♂	3.8a 2.4«±»5.2	4.0a 2.5«±»5.5	2.5a 2.3«±»2.7	2.8a 2.5«±»3.1	3.9a 3.3«±»4.5
	♀	4.5a 4.2«±»4.8	4.4a 3.9«±»4.9	3.0b 2.5«±»3.5	3.3b 2.9«±»3.7	4.1a 3.6«±»4.6
L_{MW} (mg)	♂	48.9a 45.6«±»52.2	52.6a 47.0«±»58.2	45.5a 44.2«±»46.8	45.1a 42.0«±»48.2	49.0a 45.4«±»52.6
	♀	58.6a 52.7«±»64.5	57.0a 50.3«±»63.7	57.5b 54.0«±»61.0	52.5b 48.3«±» 56.7	58.0b 53.0«±»63.0
Pupal weight (mg)	♂	33.8a 31.6«±»36.0	37.1a 33.0«±»41.2	31.6a 29.6«±»33.6	30.8a 29.7«±»31.9	32.9a 30.4«±»35.4
	♀	44.6a 40.1«±»49.1	42.4a 38.6«±»46.2	42.8a 40.8«±»44.8	39.9a 37.0«±»42.8	43.8a 41.3«±»46.3

Table 6.2: Effect of thermophotoperiod in the final

instar of *C. jactatana*

* at 18 ±1°C, 75 ±5%RH

** mean «±» 95% confidence intervals

Rows followed by the same letter are not

significantly different (p>0.01)

		Thermophotoperiodic regimen*	
		LD 12:12, 22-14°C	LD 12:12, 14-22°C
n	♂	59	65
	♀	53	55
Duration of L_s (days)	♂	10.6a 10.2≪±≫11.0**	9.2b 9.0≪±≫9.4
	♀	11.0a 10.7≪±≫11.3	9.9b 9.7≪±≫10.1
Pre-L_{cw} period (days)	♂	1.9a 1.6≪±≫2.2	1.5a 1.3≪±≫1.7
	♀	1.8a 1.5≪±≫2.1	1.6a 1.4≪±≫1.8
Pre-pupal period (days)	♂	5.1a 4.5≪±≫5.7	4.8a 4.1≪±≫ 5.5
	♀	5.3a 5.0≪±≫5.6	4.8a 4.5≪±≫5.1
Latent feeding period (days)	♂	2.8a 2.5≪±≫3.1	2.0b 1.9≪±≫2.1
	♀	3.0a 2.7≪±≫3.3	2.4b 2.2≪±≫ 2.6
L_{MW} (mg)	♂	47.0a 45.3≪±≫48.7	40.0b 38.5≪±≫41.5
	♀	60.1a 57.4≪±≫62.8	52.0b 50.4≪±≫53.6
Pupal weight (mg)	♂	32.4a 31.2≪±≫33.6	27.7b 27.0≪±≫28.4
	♀	44.8a 43.3≪±≫46.3	39.7b 38.6≪±≫40.8

Figure 6.1: Effect of photoperiod on the final instar

duration of *C. jactatana*

Vertical bars represent 95% confidence intervals

(See Table 6.1 for sample size)
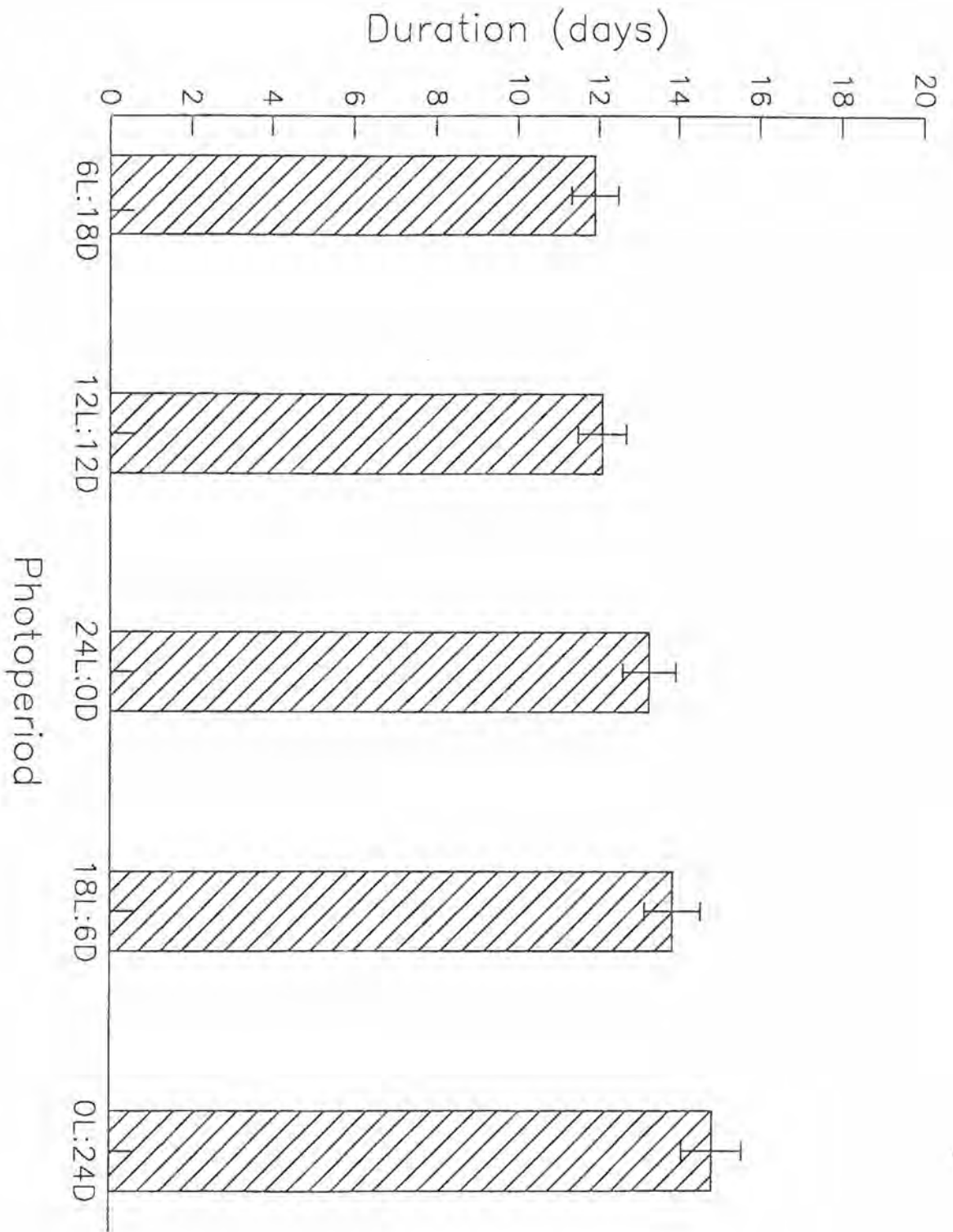
CHAPTER / 7

POSITIVE ARTIFICIAL ASSORTATIVE SELECTION FOR RATE OF DEVELOPMENT
AND PUPAL WEIGHT IN *'Cnephasia' jactatana* (Walker) (Lepidoptera:
Tortricidae) - A GENETIC TOOL FOR QUALITY IMPROVEMENT.

7.1 ABSTRACT

Artificial assortative selection for fast and slow rate of
pupation over three successive generations in *'Cnephasia' jactatana*
produced two distinct 'strains'. A 'fast strain' that pupated 2 days
earlier than the 'slow strain'. Assortative selection for pupal
weight was asymmetrical with the the 'light' selection segregating
more than the 'heavy' selection. The larval critical weight (L_{CW}),
the larval maximum weight (L_{MW}) and the latent feeding period were
not affected by any of the assortative selections. 'Slow' and
'light' selections had longer pre-pupal periods than 'fast','heavy'
or the laboratory population. Fecundity was reduced in females from
'slow' and 'light' selections. Assortative selection in insects is
discussed as a possible genetic tool for overall quality improvement.

7.2 INTRODUCTION

Experimental attempts of artificial genetic manipulation for improvement of insect performance and management are well documented (Bell *et al.* 1955; Bell, 1963; Cale and Gowen, 1956; Cale and Rothenbuhler, 1975; Bartlett, *et al.* 1966; Enfield *et al.* 1966; 1969; Enfield, 1972; Mackauer, 1972; Hoy, 1976; 1979; Collins, 1984). The incorporation of genetic methods for quality improvement is, however, not widely practiced with exception of the commercial rearing of silkworm (Tazima, 1964) and the honeybee (Kerr, 1974). Knowledge from the genetical research on *Drosophila* and *Tribolium* has so far been used as a source for data on mating systems and gene action (Sokolof, 1974; see also Hoy, 1976, for review).

The overall performance and quality of adult holometabolous insects is dependent on size which correlates with pupal weight (Kester and Smith, 1984; Engelmann, 1984; Slansky and Scriber, 1985). It has been shown that pupal weight is dependent on the level of the larval maximum weight (L_{MW}), that a larva attains in the final instar (see chapter 3). The level of L_{MW} is dependent on the quality of nutrition and amount of food ingested during the latent feeding period (period between achieving a larval critical weight (L_{CW}) and the L_{MW}. In the black lyre leafroller, '*Cnephasia*' *jactatana*, the L_{CW} is attained after 1-3 days of feeding at 20°C and is thought to be linked to a mechanical-stretch process

that initiates steps that ultimately result in the secretion of the juvenile hormone (see Bhaskaran *et al.* 1980; Nijhout, 1981; Jones *et al.* 1981 on the tobacco hornworm, *Manduca sexta*). L_{CW} is not influenced by nutrition, temperature or photoperiod (see chapter 4, 5 and 6).

Assortative mating is a system where the mating pairs are phenotypically more closely identical than they would be if the pairs were chosen at random from a population (Gianola, 1982; Fernando and Gianola, 1984). Positive assortative mating occurs when the mates are of similar phenotype (and thus probably of similar genotype) while negative assortative mating occurs when mates are selected from dissimilar phenotypes (Edwards 1977). Positive assortative mating has been shown to be of use in genetic improvement of insects by rapid selection of preferred features (Englert and Bell, 1970; King and Dawson, 1972; Mwenya *et al.* 1986).

Experiments were devised to investigate the effect of positive artificial assortative selection for 1) rate of development and 2) pupal weight, on the L_{CW}, latent feeding period, L_{MW} and reproductive performance of *C. jactatana*. The results emphasize the utility of assortative selection as a genetic tool for quality improvement.

7.3 MATERIALS AND METHODS

Insects used in the experiment were derived from a laboratory population maintained at 20 ±1°C, 75 ±5%RH and LD 18:6 for 9 generations under a random mating system (see chapter 2, Figure 2.1) on a general purpose diet (GPD, Singh, 1983; see appendix A.1 for details of rearing and Table A.4 for diet composition.

1) Assortative selection for rate of development were performed by mating adults eclosing together either from the first or last pupae formed from five group rearing units (each containing approximately 125 insects, see appendix Table A.2 for details of the unit). The adults (n=10 pairs for each category) were mated in polythene group oviposition units (4 replicates, see appendix Table A.2 for details of unit construction). Eggs were collected after a fortnight and a random sample of 100 neonate larvae were individually inoculated into perspex test tubes containing GPD and reared until pupation. Male adults eclosing from the first larvae to pupate were again mated with females from the first pupae ('fast strain'). Adults eclosing from the last pupae were paired similarly ('slow strain') and the procedure was repeated over three successive generations (G_1-G_3).

2) Assortative selection for pupal weight was conducted for an equal sample size in a similar manner, adults (male and female) eclosing from the heaviest pupae measured were crossed ('heavy strain') as were males and females from pupae of the lightest weight measured ('light strain'). The procedure was also repeated for three successive generations (G_1-G_3).

To evaluate the impact of these selections on the life cycle, neonate larvae (n=100 for each selection) were obtained after the third generation of assortative selection and reared to the adult stage. Observation was maintained for complete larval and pupal development. Male adults eclosing were maintained until death at $15 \pm 1°C$ to obtain data on longevity. Adult females were selected randomly (n=20 for each selection) and mated at $15 \pm 1°C$, $75 \pm 5\%RH$, LD 16:8 with average sized males (eclosing from pupae of about 30mg) from the laboratory population and data of female longevity and fecundity obtained.

To investigate the effect of assortative selections on the L_{CW}, neonate larvae were obtained after the third generation of respective assortative selection and were reared until moult into fifth instar (L_5, day 0). Since L_{CW} varies with size (measured as head capsule width), only L_5 larvae of medium size (head capsule width 1.18-1.20mm) were transferred to plastic vials (40 X 100mm)

after feeding for 12-24 hrs after moult and starved for 20 days
(n=30 for each selection of 'first' 'slow' 'heavy' and 'light').
Pupae formed were sexed and weighed. Larvae not pupated at the end
of the 20 days were transferred to rearing test tubes containing GPD
diet and reared until pupation whereupon their sex was determined.

To determine the effect of assortative selections on the
latent feeding period, L_{MW}, pupal and adult weight, L_5 larvae of
medium size from the four selections (n=60 for each selection) were
transferred to plastic vials containing 1.5g of GPD diet on day 0.
Individual larvae were weighed at 24 hr intervals until pupation.
Pupae were sexed and weighed then incubated until eclosion. Eclosing
adults were also weighed and their sex confirmed.

Rearing was carried out at 20 ±1°C, 75 ±5%RH, LD 18:6.
Larvae, pupae and adults were weighed using a Shimadzu electronic
balance AEL-160 with an accuracy of ±0.1mg. A control was set up for
each experiment from a random sample of the laboratory population.

7.4 RESULTS

7.4.1 *Effect of positive assortative selections on the life cycle*

Effects of the assortative selections on the life cycle after
three generations are summarized in Table 7.1. 'Fast strain' had an

overall faster development in the five larval instars. Although the 'slow strain' selection had longer developmental durations in the five instars, the instar durations were not significantly different from the random mated control ($p > 0.01$). Mean pupal weights for both sexes of the 'slow strain' selection were significantly less than of the three other strains and the control. Pupal periods were not affected by assortative selection. Adult longevity was longest in the 'fast strain' selection.

Selection for fast and slow rate of development did not have a significant effect on the pupal weight in the three generations (Figure 7.1). The 'heavy strain' selection had a higher mean pupal weight of males in the first generation. The 'light strain' selection had consistently lower pupal weights in the three generations.

Figure 7.2-7.5 display distribution frequencies of pupation in days from neonate stage for the four selections. Selecting for fast rate of development and high pupal weight had the effect of shifting pupation peaks to the first two days of pupation in both sexes (Figure 7.2 and 7.4). The 'slow strain' selection shifted the female mode from day 34-35 to day 40 while 'light strain' selection shifted the mode in both sexes from day 32 to day 38 (Figure 7.3 and 7.5).

7.4.2 *Effect of positive assortative selection on the* L_{CW}

The L_{CW} for medium sized larvae of 1.18mm (male) and 1.20mm (female) after 3 generations of assortative selection was 31.1mg and 36.8mg for 'fast strain' selection; 28.5mg and 37.2mg for 'slow strain' selection; 30mg and 36.3mg for 'heavy strain' selection and 28.5mg and 37.0mg for 'light strain' selection for males and females respectively. The controls from the random mated laboratory population had L_{CW} of 28.3mg for males and 36.7mg for females. The results indicate that assortative selections did not affect the L_{CW}.

7.4.3 *Effect of positive assortative selection on the latent feeding period and* L_{MW}

The effect of the assortative selections on developmental durations in the final instar are set in Table 7.2. In analysing the data, L_{CW} was considered to be 29.0mg for males and 36.0mg for females. Selection had no effect on the latent feeding period or the L_{MW} in any of the four selections. The 'slow' and the 'light' selections had significantly longer pre-pupal periods (p<0.01). However, the decrease in weight from L_{MW} to pupa (D_P) for the 'slow' and 'light' selections were significantly higher for both sexes from the other selections at p<0.01. Decrease in weight from pupa to adult was not significant in any of the selections.

7.4.4 *Effect of positive assortative selections on the reproductive*

performance

The predicted reproductive response in Table 7.1 was

calculated by use of an equation obtained from regressing fecundity

on pupal weight. Pupal quality indices are the ratio of pupal weight

to the P_{CW} (chapter 3 for details). Observed fecundity of both

'slow' and 'light' selections were significantly less than 'fast',

'heavy' and controls ($p < 0.01$) .

7.5 DISCUSSION

Selective breeding of any type will usually lead to

inbreeding, genetic drift and loss of beneficial genetic variation

(Bush, 1979). Breese (1956) discussed genetical consequences of

assortative mating as a process that may establish internal

chromosomal balance leading to success in breeding. Assortative

mating was selected as the breeding system for evaluation in *C.*

jactatana as it has been reported to cause less increase in

homozygosity than other types of inbreeding (Crow and Felsenstein,

1968).

Working on the selection for pupation time of *Tribolium*

castaneum, Englert and Bell (1970) suggested that phenotypic

differences in pupation time could reflect variation in hormonal

activity manifested in growth and development. Bradley and Saunders

(1986) found that in the flesh fly, *Sarcophaga argyrostoma*, the 'fast' and 'slow' selection only differed in the interval of larval wandering to pupariation, other aspects of development being identical. Bradley and Saunders held the view that 'fast' or 'slow' selection operated on genes regulating aspects of the release prothoracicotropic hormone (PTTH).

In *C. jactatana*, artificial selection for larval rate of development causes an immediate divergence (within one generation) for fast and slow development. Unlike *S. argyrostoma*, there are differences in both sexes in almost all larval instars, with the 'slow' selection taking longer to develop in each instar than 'fast' selection. The immediate divergence of the 'fast' selection compared to the 'slow' selection suggests that the selection of fast rate of development is easier to achieve. This finding is in agreement with data from *S. argyrostoma* and may also indicate either a smaller number of polygenic alleles for fast development than for slow, or a more homogeneous set of alleles. The finding also supports Englert and Bell (1969; 1970) who found that the effectiveness of shift for early pupation in *Tribolium* is 1+ greater for early than for late pupation. It is possible therefore, that asymmetry of heritability exits for rate of larval development.

Artificial selection for pupae of high pupal weight is only significant within the first generation males. On the other hand, the selection of light pupae is maintained in both sexes in the three generations. It appears that the selection for pupal weight in *C. jactatana* is also asymmetrical with the selection for light pupae being more effective than for heavy pupae. There is no apparent relationship between developmental time, pupal weight or size (head capsule width). Unlike *Tribolium* (King and Dawson 1972; Bell and Moore, 1972), pupal weight in *C. jactatana* is likely to be of medium heritability. Rate of development is probably a more heritable trait.

The results do not indicate any significant differences for the L_{CW}, latent feeding period and L_{MW} of similar sized larvae from the four assortative selections. The pre-pupal stage of both 'slow' and 'light' selections are however, longer than in the 'fast', 'heavy 'or controls. D_P is also significant in both sexes of the 'slow' and the 'light' selections. These results suggest that differences between the rates of development may not be due to a hormone system initiating metamorphosis (juvenile hormone or PTTH) but rather to processes that lead to pupal ecdysis after attainment of the L_{MW} (i.e. after release of PTTH). It is probable that the low pupal weights associated with 'slow' and 'light' selections is linked to the prolonged pre-pupal period leading to a higher D_P (see also Hinks and Byers, 1976, work on aestivating classes of the genus *Euxoa*). Of greater interest, though, is the fact that the L_{CW} is

not affected by any of the four selections. This result emphasizes the likelihood of a mechanical-stretch phenomena operating as a threshold to pupation (Nijhout, 1981; see also chapters 3, 4, 5, and 6).

Reproductive performance of females was poorest in assortations for 'slow' and 'light' quantitative characters. It is not clear why fecundity of these selections was low although it may be related to the long pre-pupal and higher D_P these selections had. It is clear, however, that selecting for slow rate of development does not contribute to an increase in pupal weight or reproductive performance nor does a selection of low pupal weight result in an increase in reproductive performance. In insects generally, it has been shown that large females produce comparatively larger clutches of eggs than small females (Thornhill and Alcock, 1983). Large size is well maintained in selections for fast development and heavy pupal weight.

7.6 CONCLUSION

The results emphasize the utility of assortative selections for phenotypic characters as a base for overall quality improvement. The effectiveness of genetic improvement of the type discussed will depend on the genetic variability of the parental stock. More variability will slow inbreeding depression (Boller, 1972). In

practice, 'mixed' assortative models (selecting for more than one trait, e.g. females of high pupal weight mated with fast growing males) should be considered in improvement of quality since Gianola (1982) showed that 'mixed' models increase performance and genetic correlation. Although the assortative selections in *C. jactatana* were not designed to investigate genetic correlations, the results do not deny a possible genetic correlation between fast rate of development, high pupal weight and high reproductive performance. More experimentation is needed to verify the existence of such a beneficial correlation. 'Fast' and 'heavy' selections are superior to 'slow' and 'light' selections as confirmed by female reproductive performance. Slow development and low pupal weight are negative quantitative traits resulting in reduced reproductive performance.

Table 7.1 Effect of three generations of positive

assortative selections on the life cycle

of *C. jactatana*

* at $20 \pm 1°C$, $75 \pm 5\%$RH

** mean «$\pm$» 95% confidence intervals

Rows followed by the same letter are not

significantly different ($p > 0.01$)

		'Fast strain'	'Slow strain'	'Heavy strain'	'Light strain'	Control
		Assortative selections*				
N	♂	50	46	36	23	46
	♀	41	51	39	19	43
Duration of L₁ (days)	♂	7.0a 6.7«±»7.3**	8.8b 7.8«±»9.8	8.0b 7.3«±»8.7	8.3b 7.6«±»9.0	8.3b 7.5«±»9.1
	♀	7.4a 6.9«±»7.9	8.5b 8.1«±»8.9	7.7b 7.1«±»8.3	8.5b 7.4«±»9.6	8.2b 7.3«±»9.1
Duration of L₂ (days)	♂	4.4a 4.2«±»4.6	5.2b 4.9«±»5.5	5.1b 4.9«±»5.3	4.9b 4.7«±»5.1	6.4c 5.7«±»7.1
	♀	4.5a 4.3«±»4.7	5.1b 4.7«±»5.5	5.0a 4.8«±»5.2	5.0a 4.7«±»5.3	5.8b 5.2«±»6.4
Duration of L₃ (days)	♂	4.4a 4.1«±»4.7	4.9a 4.5«±»5.3	4.7a 4.5«±»4.9	4.7a 4.5«±»4.9	4.5a 4.3«±»4.7
	♀	4.4a 4.2«±»4.6	5.2b 4.9«±»5.5	4.9b 4.7«±»5.1	4.9b 4.8«±»5.0	5.0b 4.6«±»5.4
Duration of L₄ (days)	♂	4.9a 4.7«±»5.1	5.9b 5.4«±»6.4	5.3b 5.0«±»5.6	5.5b 5.3«±»5.7	4.8a 4.6«±»5.0
	♀	5.1a 5.0«±»5.3	6.3b 6.0«±»6.6	5.6b 5.4«±»5.8	5.7b 5.5«±»5.9	5.3a 5.0«±»5.3
Duration of L₅ (days)	♂	12.9a 12.0«±»13.8	13.9b 13.4«±»14.4	12.5a 11.9«±»13.1	14.7b 13.5«±»15.9	11.7a 10.9«±»12.
	♀	13.5a 13.2«±»13.8	14.6a 14.0«±»15.2	13.8a 13.1«±»14.5	13.2a 11.8«±»14.6	12.8a 11.9«±»13.
Head capsule width of L₅ (mm)	♂	1.27a 1.25«±»1.29	1.25a 1.22«±»1.28	1.24a 1.21«±»1.27	1.12b 1.10«±»1.14	1.19c 1.17«±»1.2
	♀	1.31a 1.30«±»1.32	1.29a 1.27«±»1.31	1.30a 1.29«±»1.31	1.19b 1.15«±»1.23	1.24c 1.23«±»1.2
Mean larval period (days)	♂	34.5a 33.4«±»35.6	40.3b 39.2«±»41.5	36.3a 35.3«±»37.3	38.3a 37.2«±»39.4	36.9a 36.0«±»37.
	♀	35.9a 35.4«±»36.4	38.6b 37.7«±»39.5	38.0b 37.0«±»39.0	37.8b 36.5«±»39.1	38.1b 37.0«±»39.
Mean pupal weights (mg)	♂	30.8a 29.5«±»32.2	30.4a 29.0«±»31.8	31.4a 29.7«±»33.1	20.1b 18.5«±»21.7	32.7a 31.3«±»34.
	♀	41.9a 40.1«±»43.7	42.1a 40.0«±»44.2	43.0a 40.8«±»45.2	31.0b 29.1«±»32.9	38.1c 37.0«±»39.
Pupal period (days)	♂	17.5a 17.2«±»17.8	17.1a 16.8«±»17.4	17.9a 17.3«±»18.5	17.0a 16.1«±»17.9	16.9a 16.4«±»17.
	♀	16.0a 15.8«±»16.2	16.2a 15.7«±»16.7	16.4a 16.0«±»16.8	16.0a 15.8«±»16.2	16.4a 15.7«±»17.
Adult longevity (days)	♂	36.9a 32.8«±»41.0	22.4b 16.8«±»28.0	28.1b 26.5«±»29.7	29.8a 25.5«±»34.1	21.3b 17.3«±»25.
	♀	33.0a 30.0«±»36.0	19.7b 12.9«±»26.5	24.2b 21.0«±»27.4	25.0b 18.7«±»31.3	25.5b 21.4«±»29.
Pupal quality index	♂	1.52 1.45«±»1.59	1.49 1.40«±»1.58	1.55 1.46«±»1.64	0.99 0.91«±»1.07	1.61 1.54«±»1.6
	♀	1.55 1.48«±»1.62	1.53 1.45«±»1.61	1.59 1.51«±»1.67	1.15 1.08«±»1.22	1.41 1.37«±»1.4
Predicted productive performance (eggs/female)		168 154«±»183	163 142«±»185	177 160«±»193	83 69«±»97	193 180«±»206
Observed productive performance (eggs/female)		275a 157«±»393	39b 17«±»93	273a 130«±»416	41b 2«±»74	228a 124«±»33?

Table 7.2 Effect of positive assortative selections

on final instar of *C. jactatana*

* at 20 ±1°C, 75 ±5%RH; Adult Longevity and

reproductive performance at 15 ±1°C, 75 ±5%RH

** mean «±» 95% confidence intervals

Rows followed by the same letter are not

significantly different (p>0.01)

		'Fast strain'	'Slow strain'	'Heavy strain'	'Light strain'	Control
N	♂	32	35	36	35	29
	♀	28	25	24	25	31
Pre-L$_{GW}$ period (days)	♂	2.5a 2.3«±»2.8""	3.0a 2.6«±»3.4	2.8a 2.5«±»3.1	2.8a 2.6«±»3.0	2.1a 1.9«±»2.3
	♀	2.5a 2.0«±»3.0	2.3a 2.0«±»2.6	2.2a 1.7«±»2.7	2.9a 2.7«±»3.1	2.2a 1.9«±»2.5
Pre-pupal period (days)	♂	6.5a 5.9«±»7.1	8.0b 7.6«±»8.4	6.6a 6.4«±»6.8	7.9b 7.3«±»8.5	6.5a 6.2«±»6.8
	♀	6.0a 5.8«±»6.2	8.5b 7.5«±»9.5	5.9a 4.9«±»6.9	7.5b 7.1«±»7.9	6.3a 5.9«±»6.7
Latent feeding period (days)	♂	4.0a 3.6«±»4.4	3.8a 3.3«±»4.3	3.9a 3.2«±»4.6	4.2a 3.6«±»4.8	3.5a 3.0«±»4.0
	♀	3.7a 3.0«±»4.4	4.2a 3.6«±»4.8	3.7a 2.6«±»4.8	3.6a 3.2«±»4.0	4.1a 3.5«±»4.7
L$_{MW}$ (mg)	♂	45.7a 43.1«±»48.3	46.0a 43.3«±»48.7	43.5a 41.6«±»45.4	41.5a 38.2«±»44.8	46.0a 43.0«±»49.0
	♀	61.5a 55.7«±»67.8	55.1a 51.3«±»63.6	55.5a 50.2«±»60.8	50.9a 48.1«±»53.7	53.7a 50.0«±»57.4
Pupal weight (mg)	♂	30.1a 29.0«±»31.2	26.8b 25.9«±»27.7	30.4a 29.9«±»31.9	23.4b 22.4«±»24.4	31.2a 29.6«±»32.8
	♀	44.3a 41.1«±»47.5	37.5b 31.1«±»37.5	42.5a 39.3«±»44.7	30.1b 28.0«±»32.2	39.8a 36.1«±»43.5
Adult weight (mg)	♂	16.2a 15.0«±»17.4	14.0b 13.5«±»14.5	16.5a 15.4«±»17.6	12.6b 11.5«±»13.7	16.9a 15.7«±»18.1
	♀	26.0a 24.5«±»27.5	19.8b 18.1«±»21.5	26.5a 22.4«±»30.6	18.1b 16.1«±»20.1	24.0a 21.8«±»26.2
D$_P$ (%)	♂	34.1a 32.7«±»35.5	41.7b 36.6«±»46.8	30.1a 28.1«±»32.1	43.6b 41.4«±»45.8	32.2a 31.1«±»33.3
	♀	28.0a 26.2«±»29.8	37.7b 34.4«±»41.0	24.4a 21.8«±»27.0	40.9b 38.2«±»43.6	25.9a 24.0«±»27.8
D$_A$ (%)	♂	64.6a 63.0«±»66.2	69.6b 68.8«±»70.4	62.1a 59.8«±»64.4	69.6b 67.1«±»72.1	63.3a 61.0«±»65.6
	♀	57.7a 54.0«±»61.4	64.1b 62.7«±»65.5	52.3a 47.9«±»56.7	64.4b 62.5«±»66.3	55.3a 52.1«±»58.5

Figure 7.1 Pupal weights over three generations of

positive assortative selection in *C.*

jactatana

a) Generation 1

Vertical bars represent 95% confidence intervals

(n: 'Fast'   selection male= 45 female= 50

    'Slow'   selection male= 33 female= 38

    'Heavy'   selection male= 54 female= 40

    'Light'   selection male= 40 female= 39
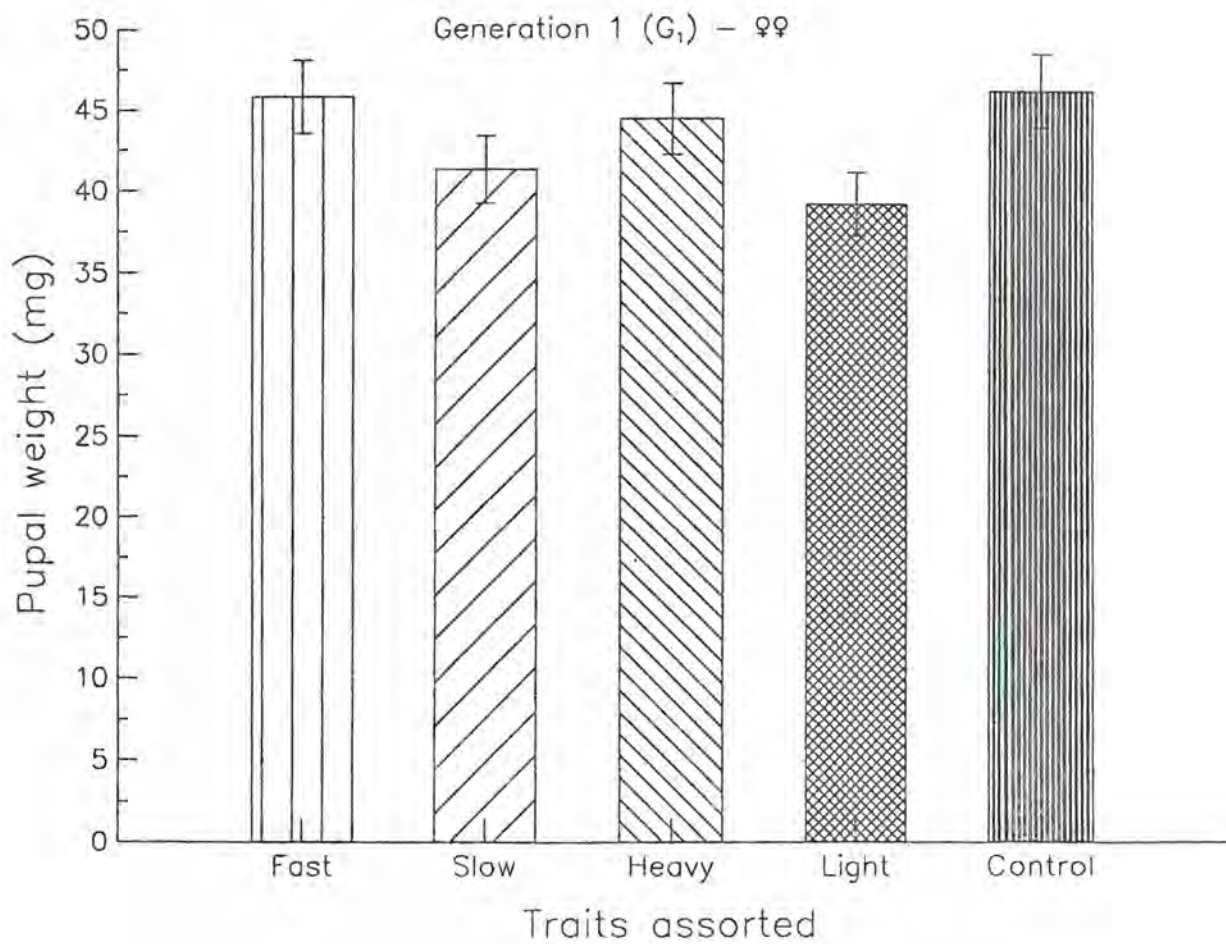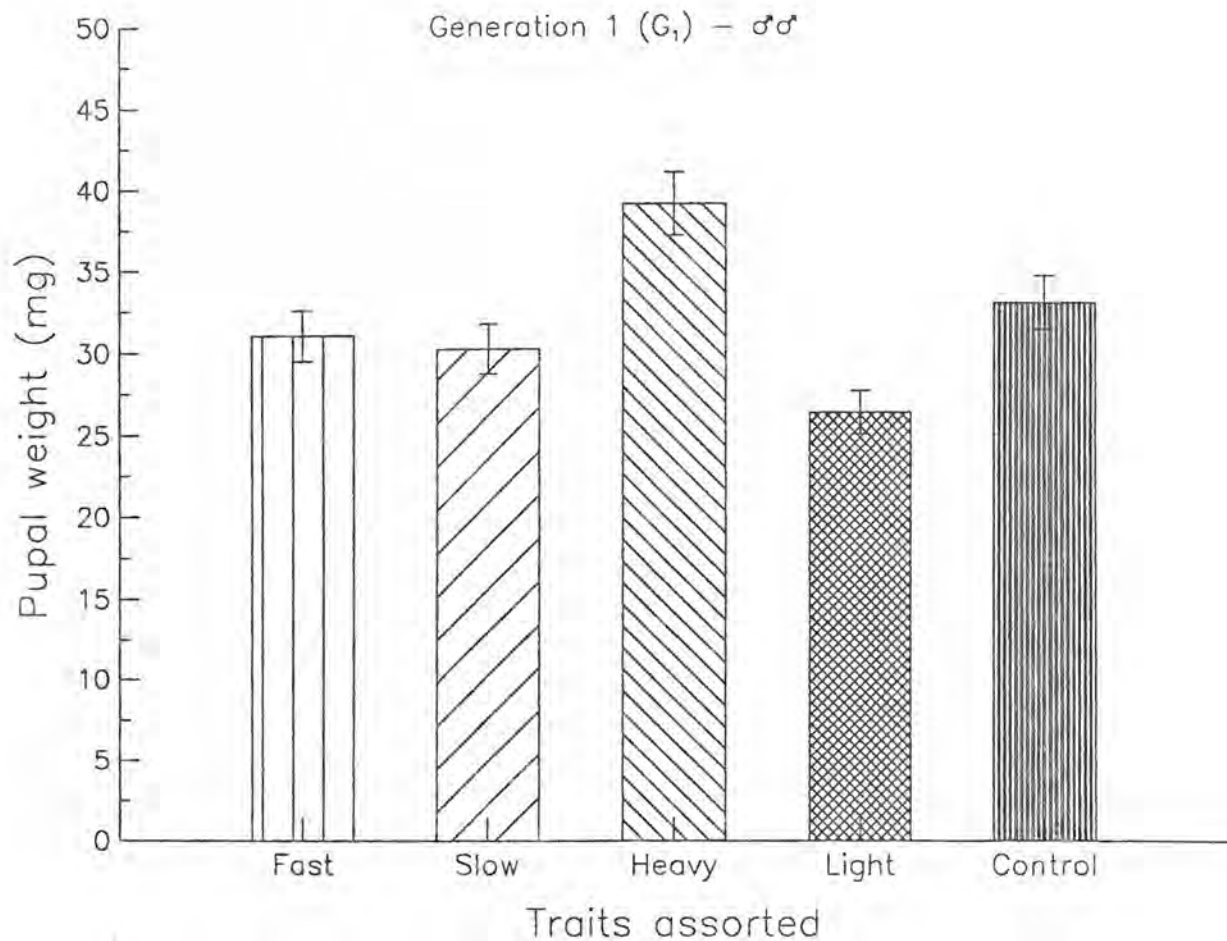
    'Control' selection male= 46 female= 43)

Generation 1 (G₁) – ♂♂

Generation 1 (G₁) – ♀♀

Figure 7.1 Pupal weights over three generations of

positive assortative selection in *C.*

jactatana

b) Generation 2

Vertical bars represent 95% confidence intervals

(n: 'Fast'   selection male= 46 female= 37

'Slow'   selection male= 49 female= 47

'Heavy'   selection male= 38 female= 47

'Light'   selection male= 38 female= 35

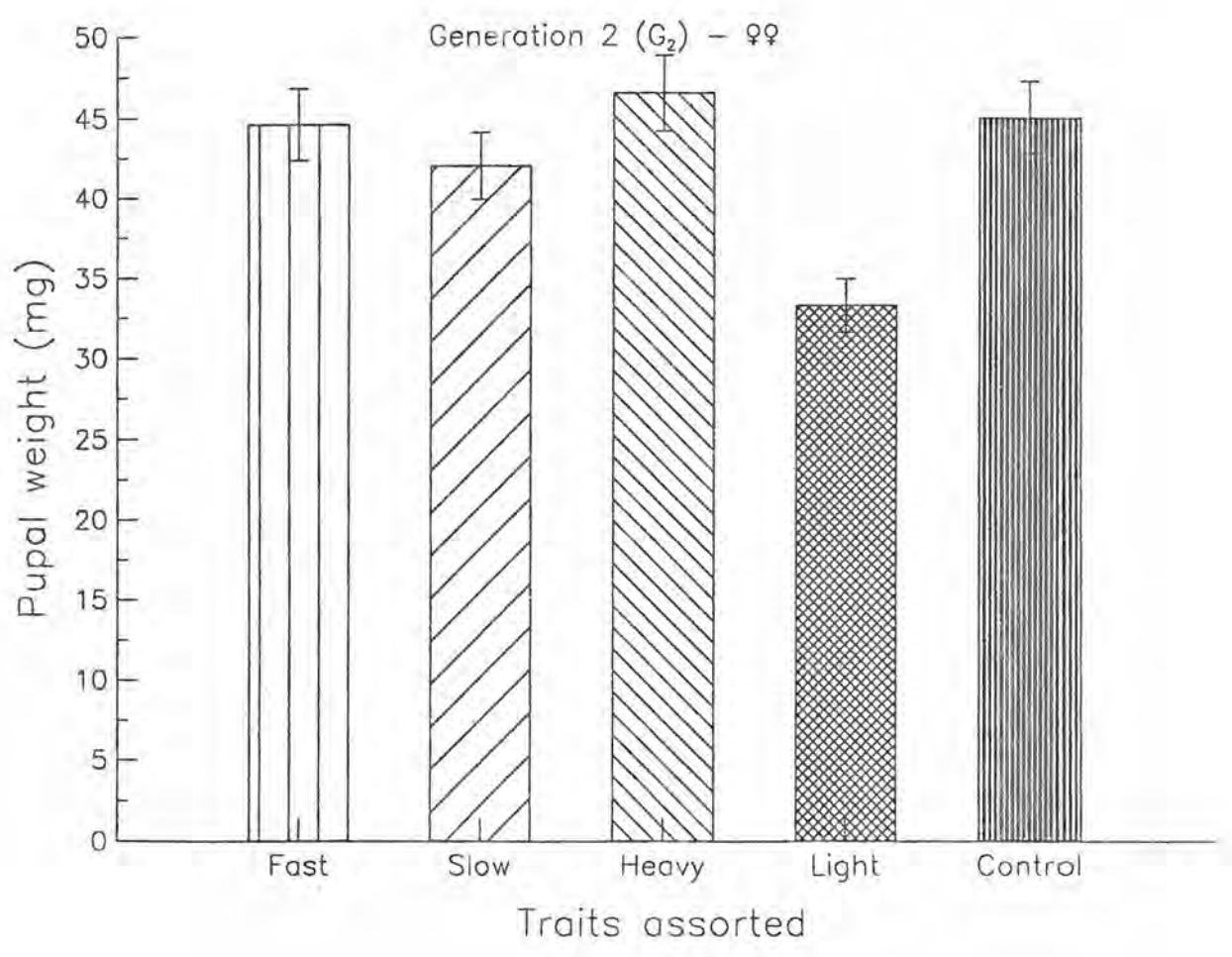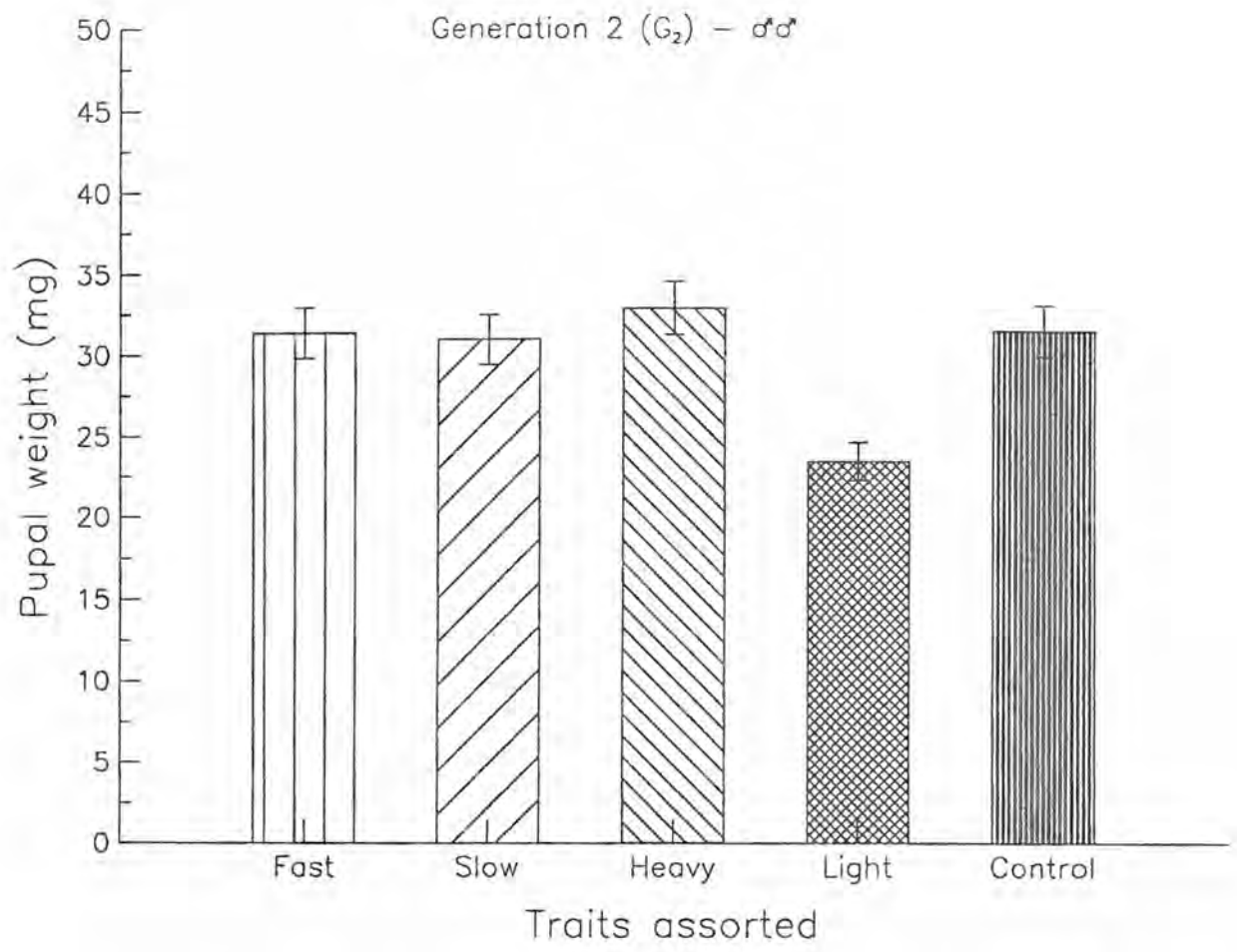'Control' selection male= 40 female= 46)

Generation 2 (G$_2$) — ♂♂

Generation 2 (G$_2$) — ♀♀

Figure 7.1 Pupal weights over three generations of

positive assortative selection in C.

jactatana

c) Generation 3

Vertical bars represent 95% confidence intervals

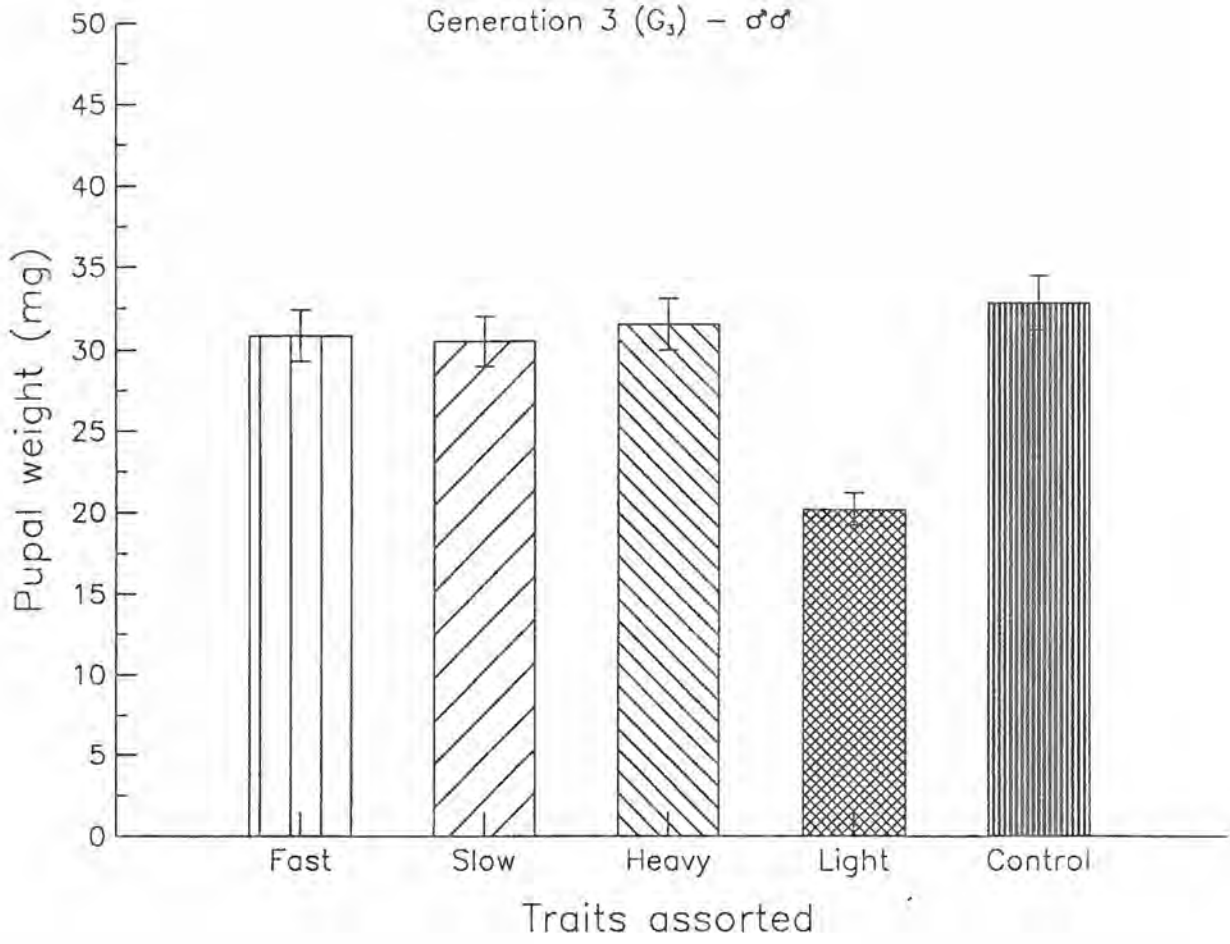(n: 'Fast'   selection male= 50 female= 41

'Slow'   selection male= 46 female= 51

'Heavy'   selection male= 36 female= 39

'Light'   selection male= 23 female= 19

'Control' selection male= 56 female= 41)
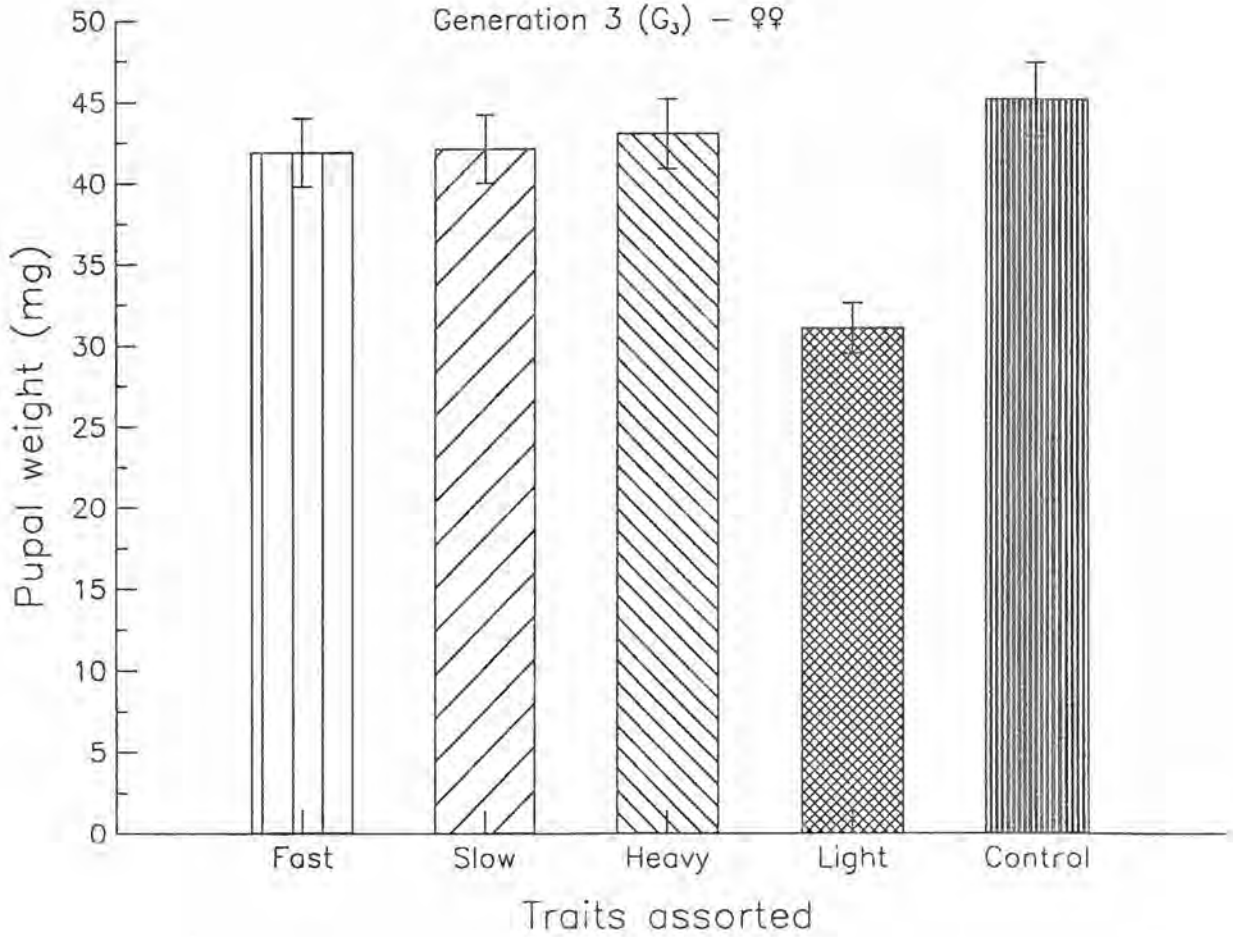
Generation 3 (G₃) — ♂♂

Generation 3 (G₃) — ♀♀

Figure 7.2 Distribution of pupation times over three

generations of assortative selection for

fast rate of development

a) Generation 1

(n: male= 45 female= 50)

GENERATION 1 (G₁) – ♂♂
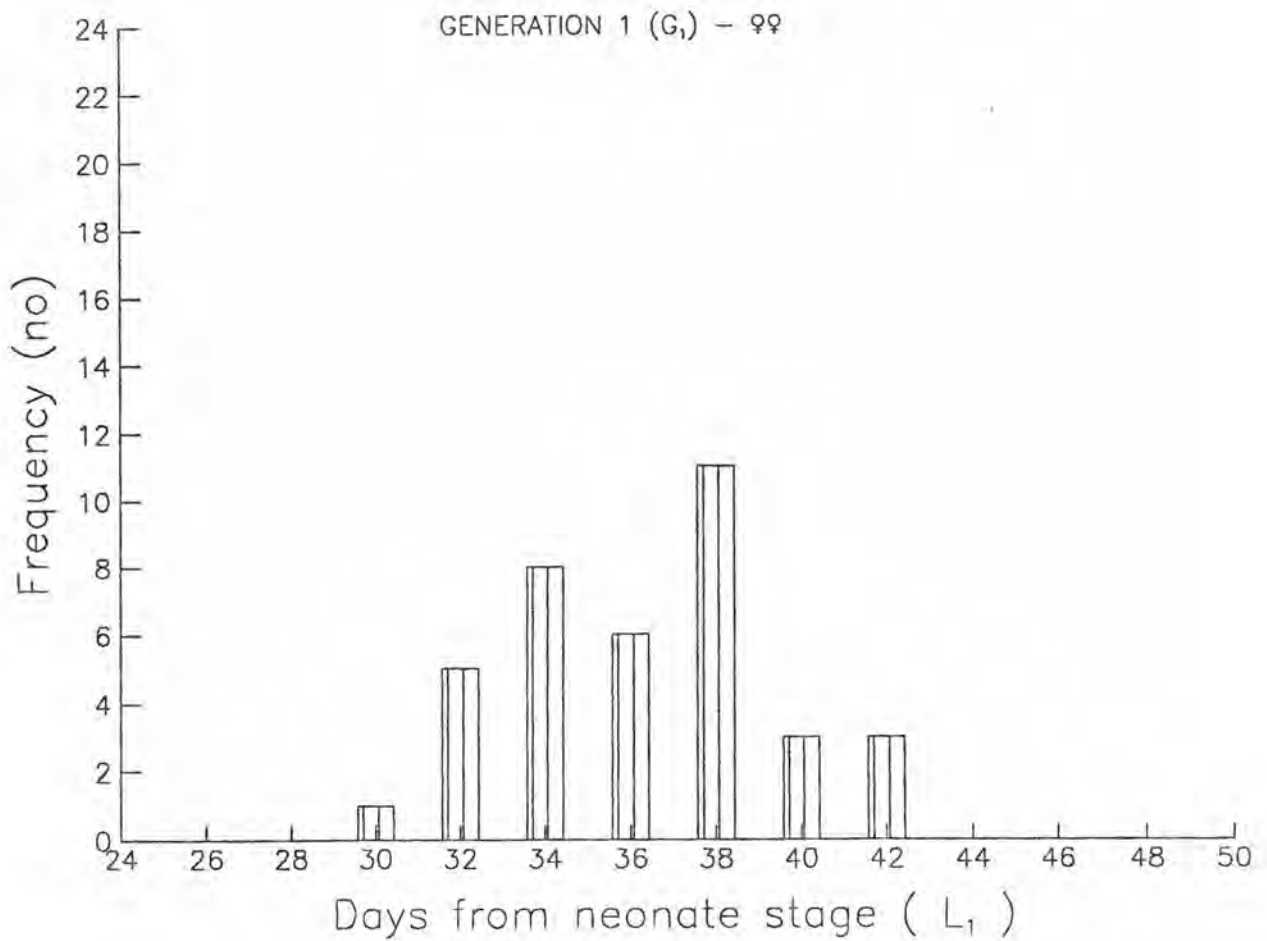
GENERATION 1 (G₁) – ♀♀

Figure 7.2 Distribution of pupation times over three

generations of assortative selection for

fast rate of development

b) Generation 2

(n: male= 46 female= 37)

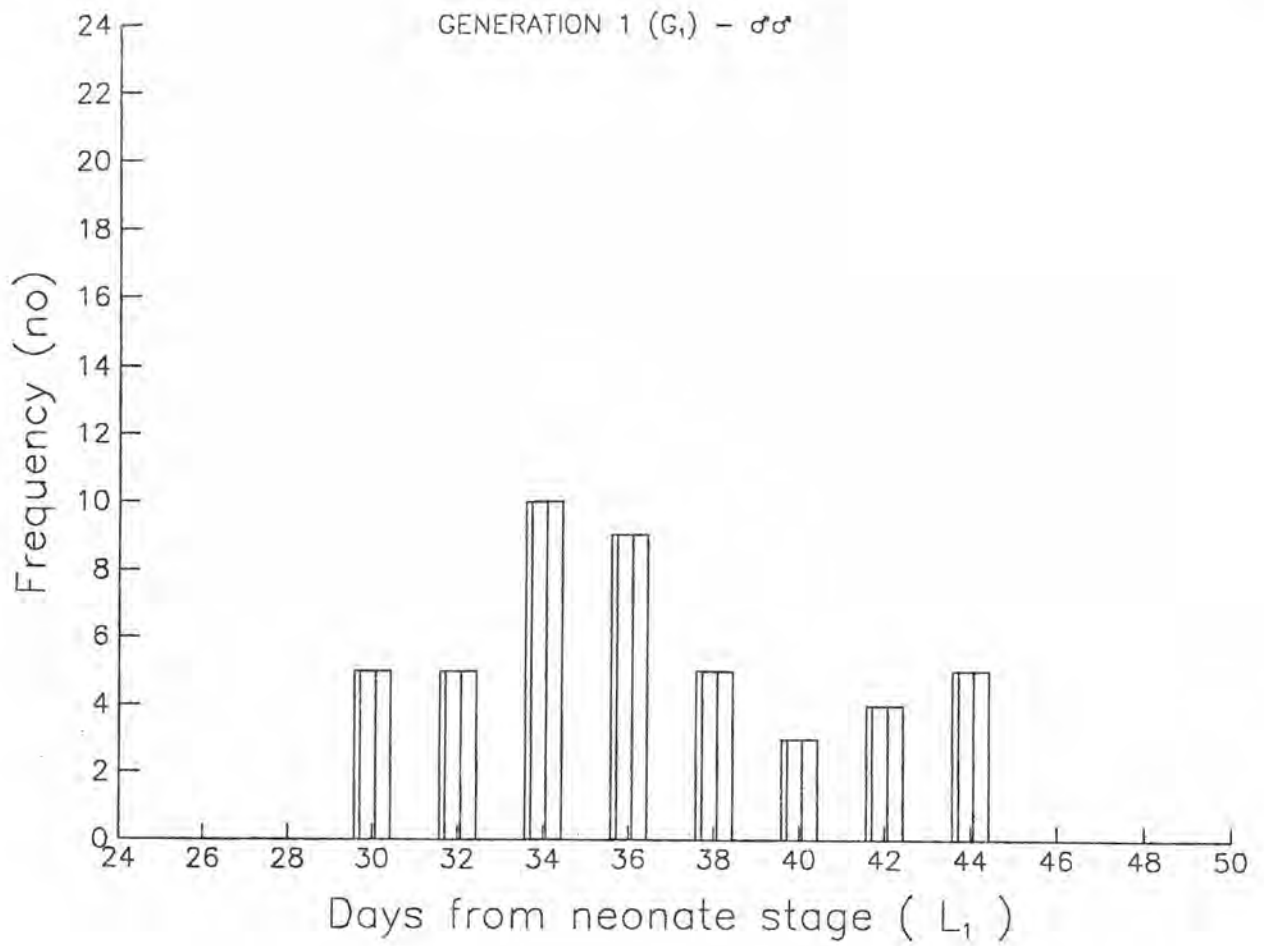GENERATION 2 (G₂) – ♀♀
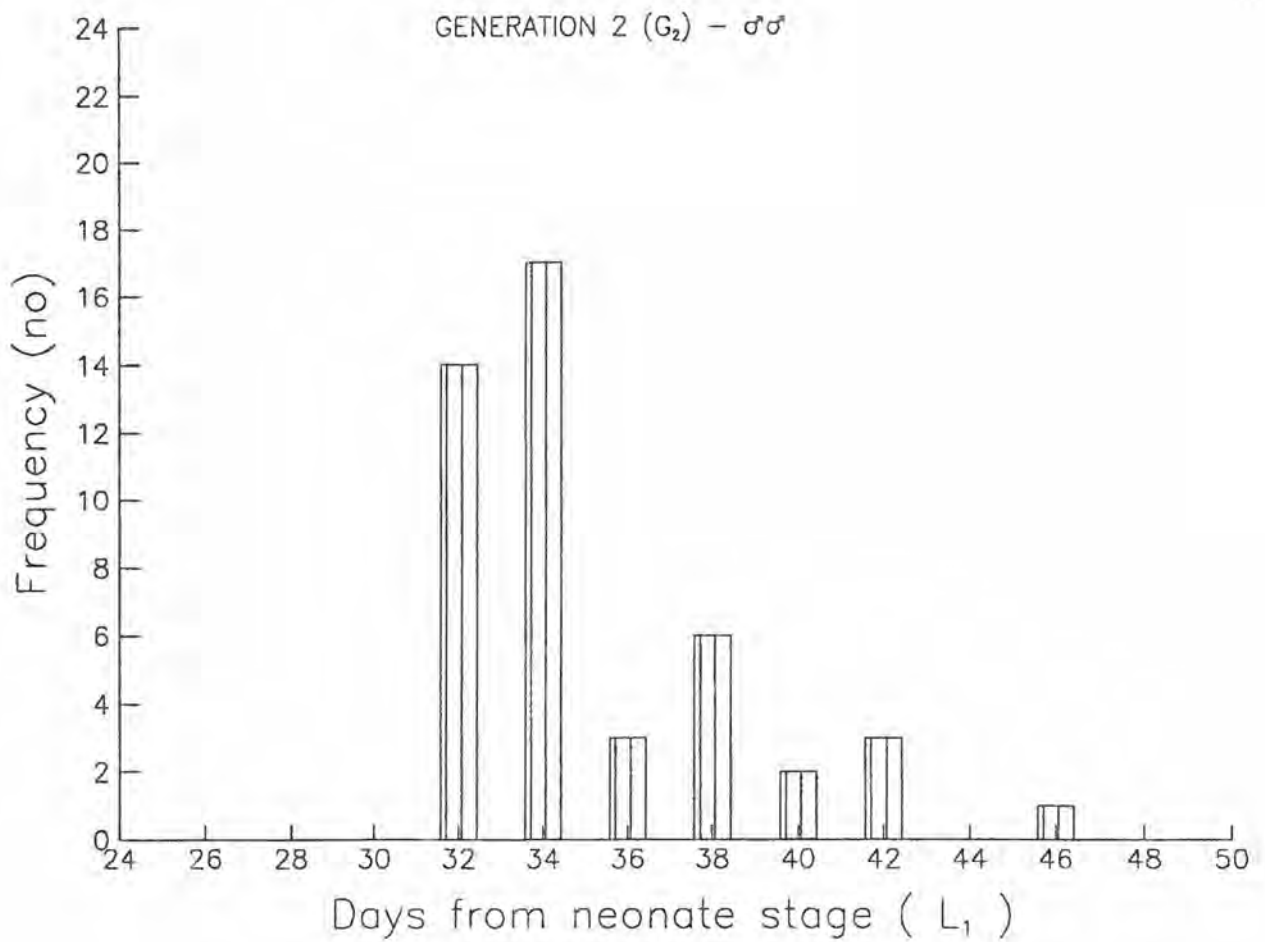
GENERATION 2 (G₂) – ♂♂

Figure 7.2 Distribution of pupation times over three

generations of assortative selection for

fast rate of development

c) Generation 3

(n: male= 50 female= 41)

GENERATION 3 (G₃) – ♂♂

GENERATION 3 (G₃) – ♀♀

Figure 7.3 Distribution of pupation times over three

generations of assortative selection for

slow rate of development

a) Generation 1

(n: male=33 female=38)

Figure 7.3 Distribution of pupation times over three

generations of assortative selection for

slow rate of development

b) Generation 2

(n: male=49 female=47)

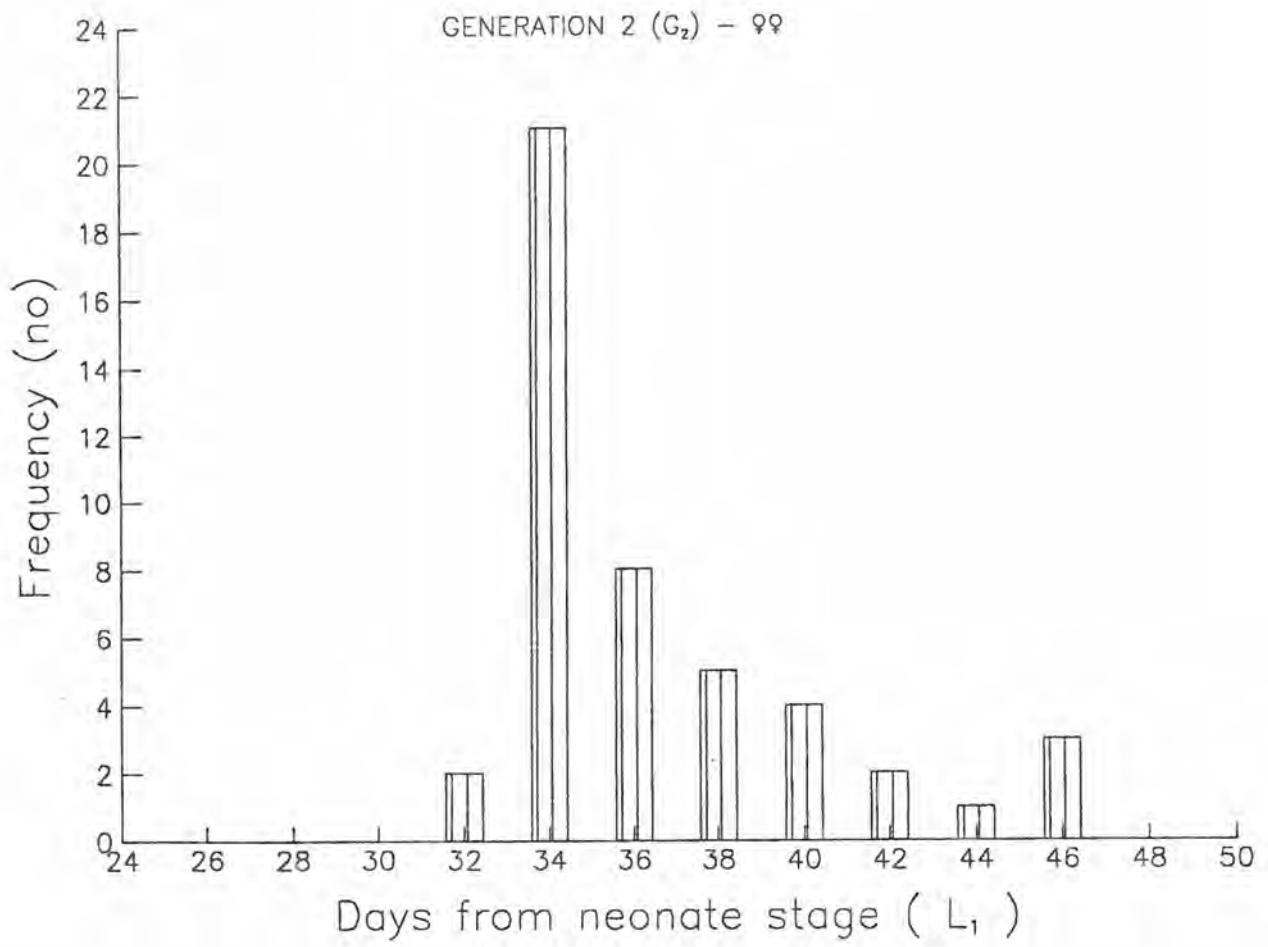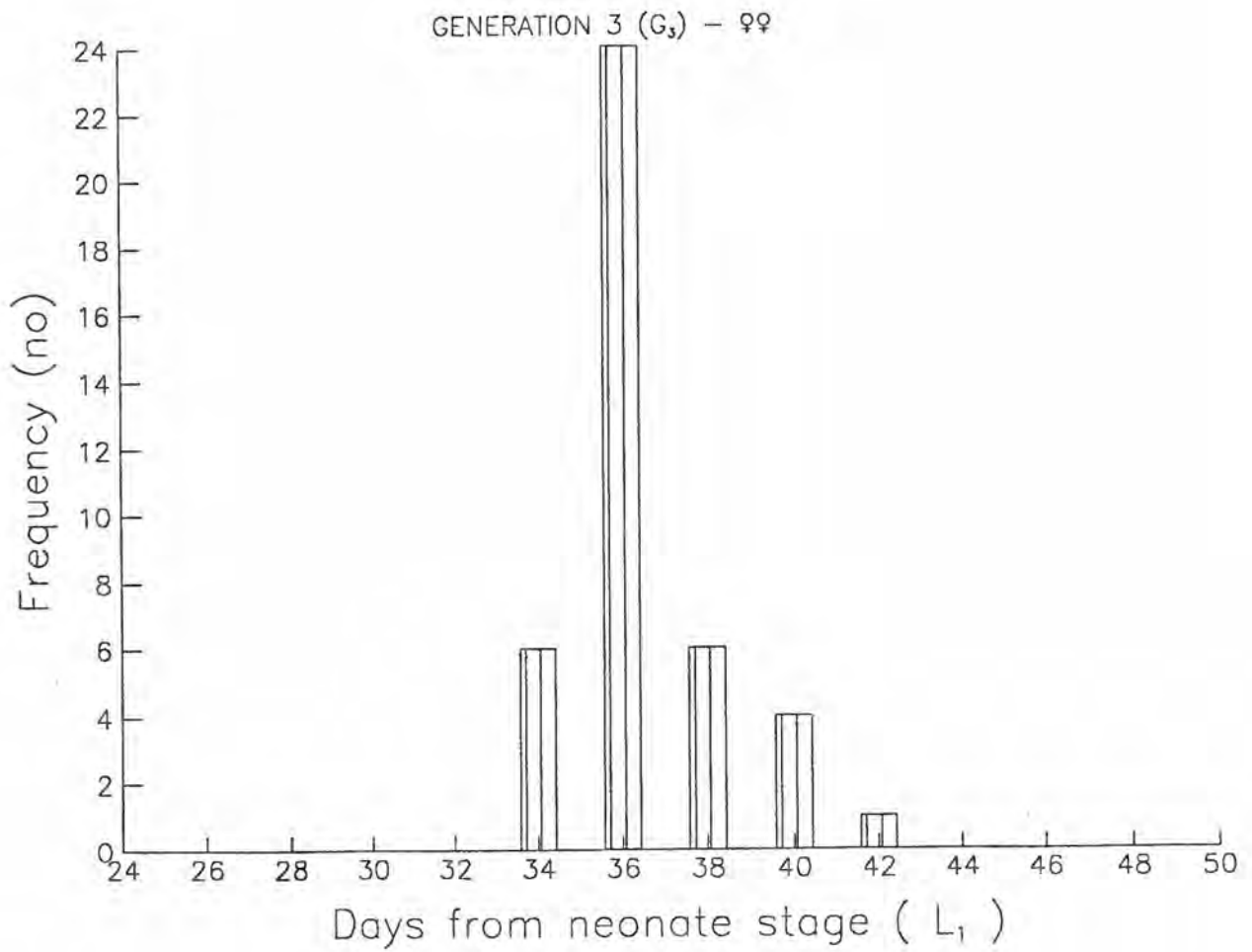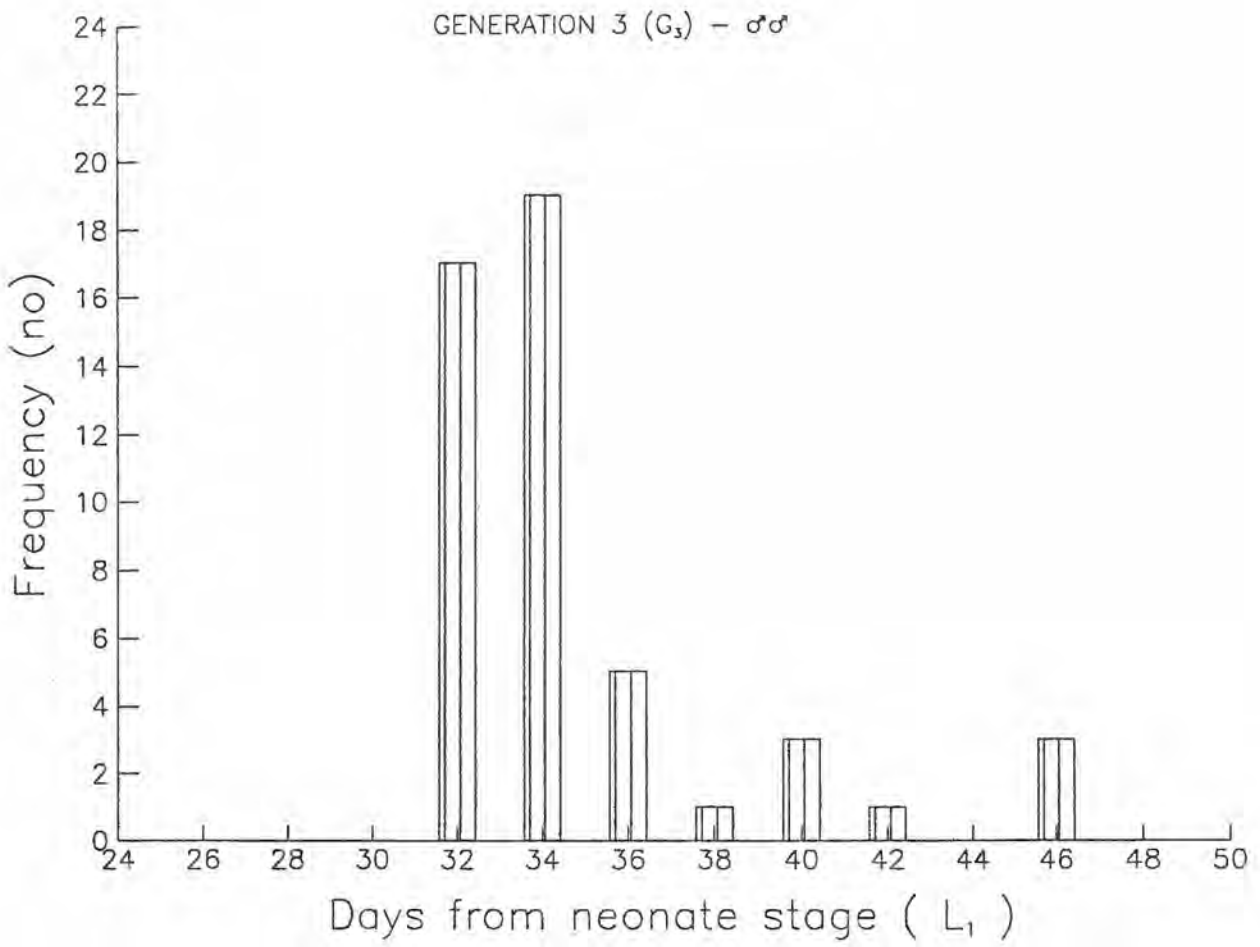GENERATION 2 (G$_2$) – ♂♂

GENERATION 2 (G$_2$) – ♀♀

Figure 7.3 Distribution of pupation times over three

generations of assortative selection for

slow rate of development

c) Generation 3

(n: male=46 female=51)

GENERATION 3 (G_3) – ♂♂

GENERATION 3 (G_3) – ♀♀

Figure 7.4 Distribution of pupation times over three

generations of assortative selection for

heavy pupal weight

a) Generation 1

(n: male=54 female=40)
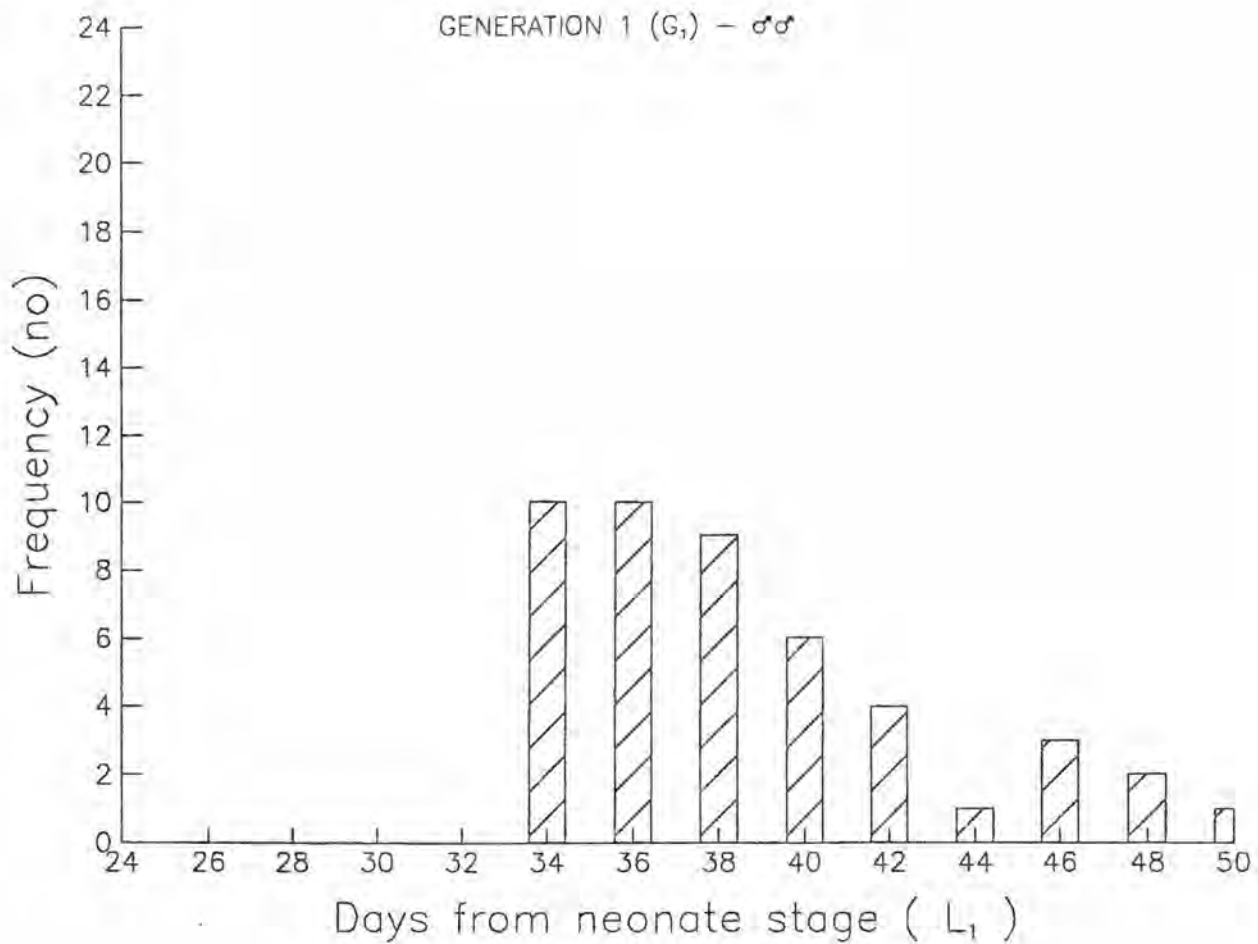
GENERATION 1 (G₁) – ♂♂

GENERATION 1 (G₁) – ♀♀

Figure 7.4 Distribution of pupation times over three

generations of assortative selection for

heavy pupal weight

b) Generation 2

(n: male=38 female=47)
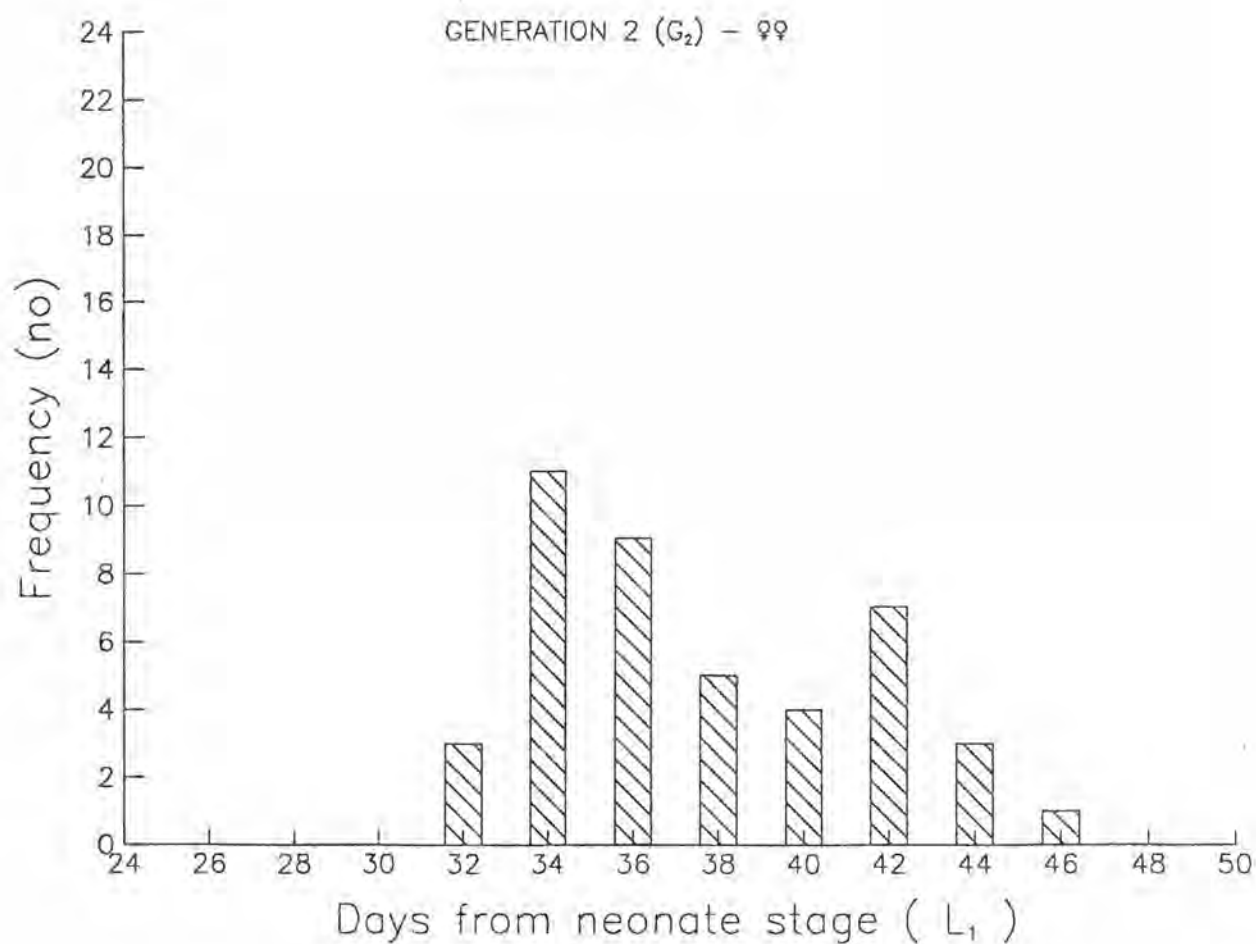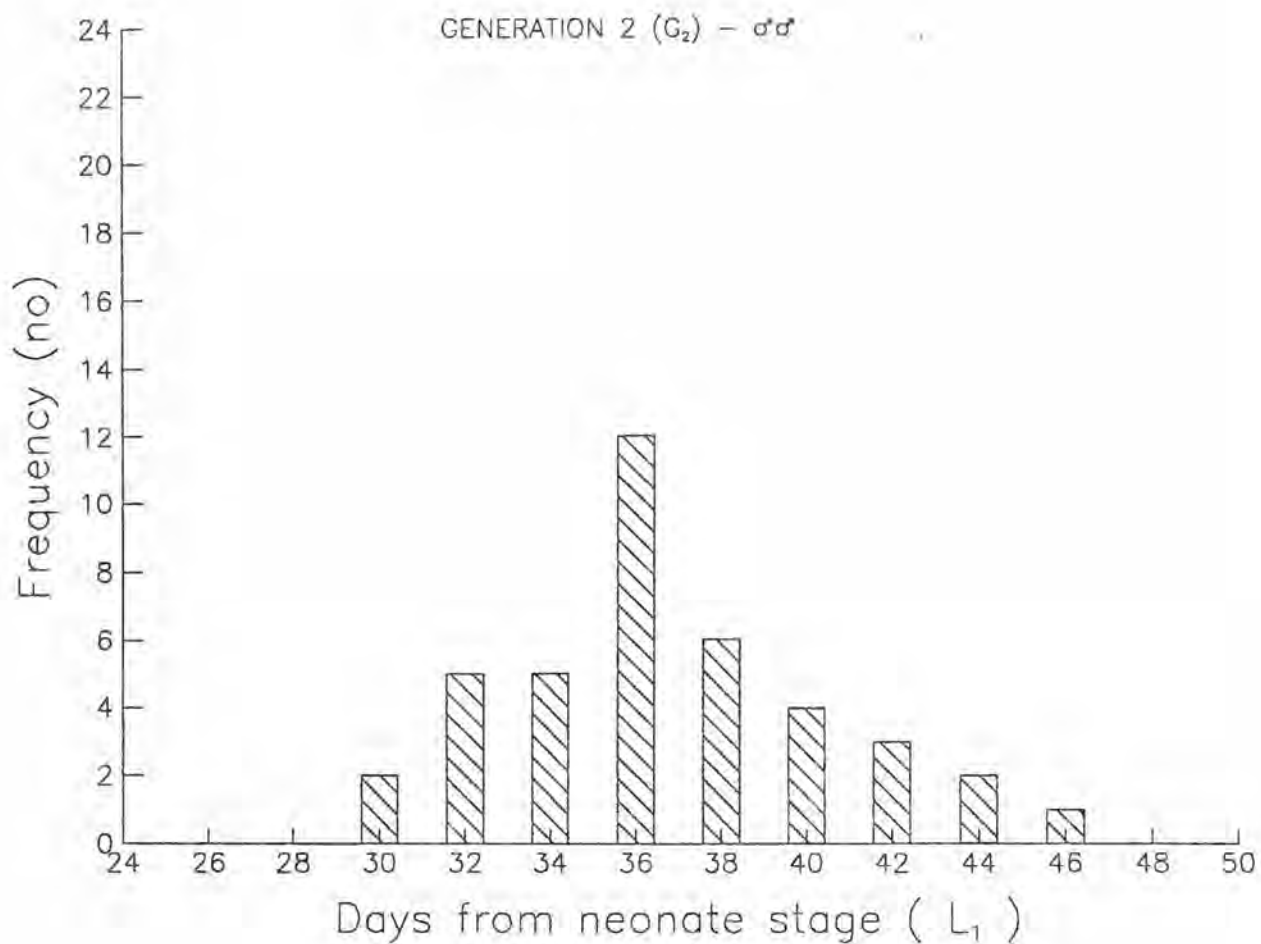
GENERATION 2 (G₂) – ♂♂

Figure 7.4 Distribution of pupation times over three

generations of assortative selection for

heavy pupal weight

c) Generation 3

(n: male=36 female=39)
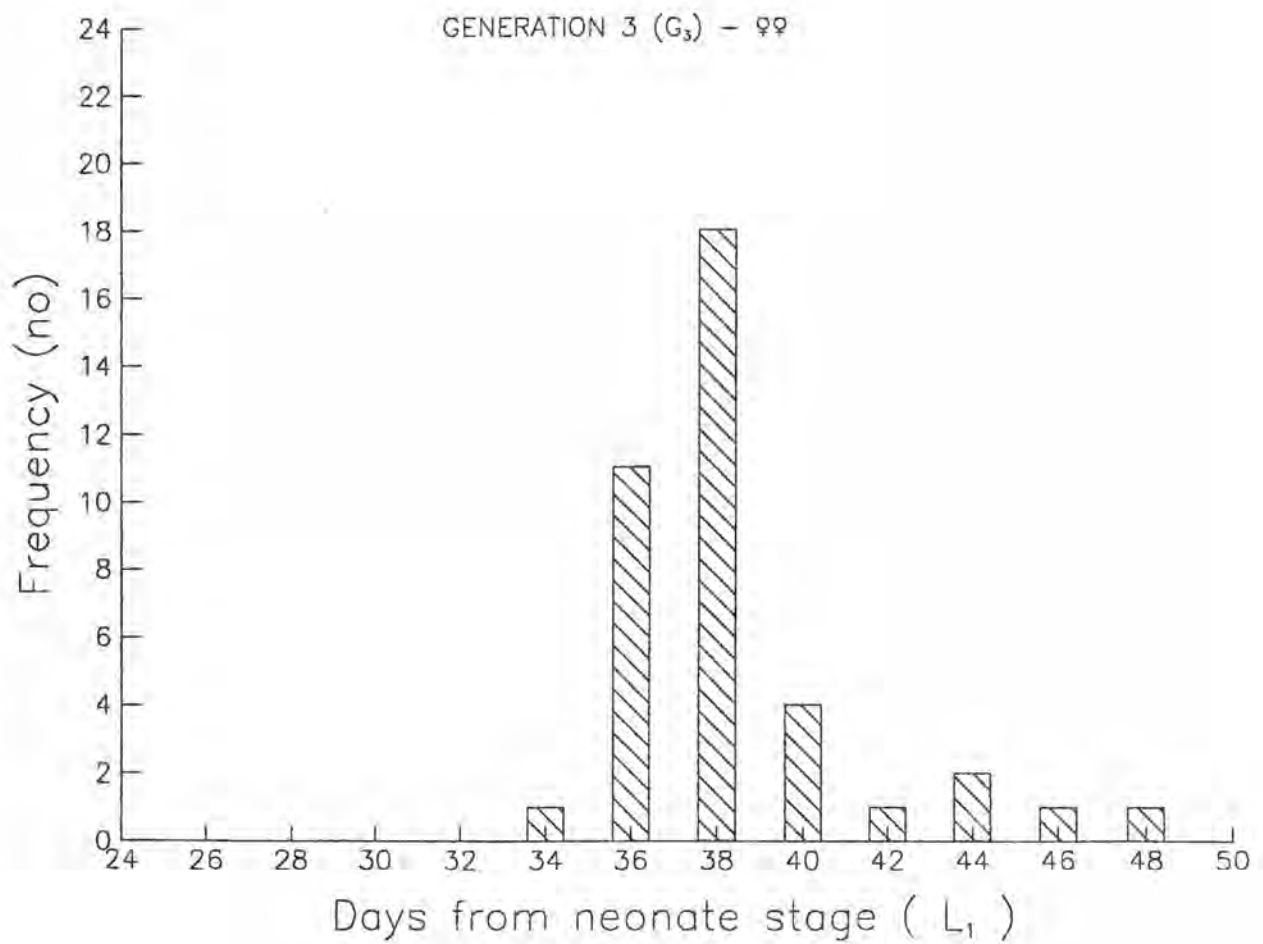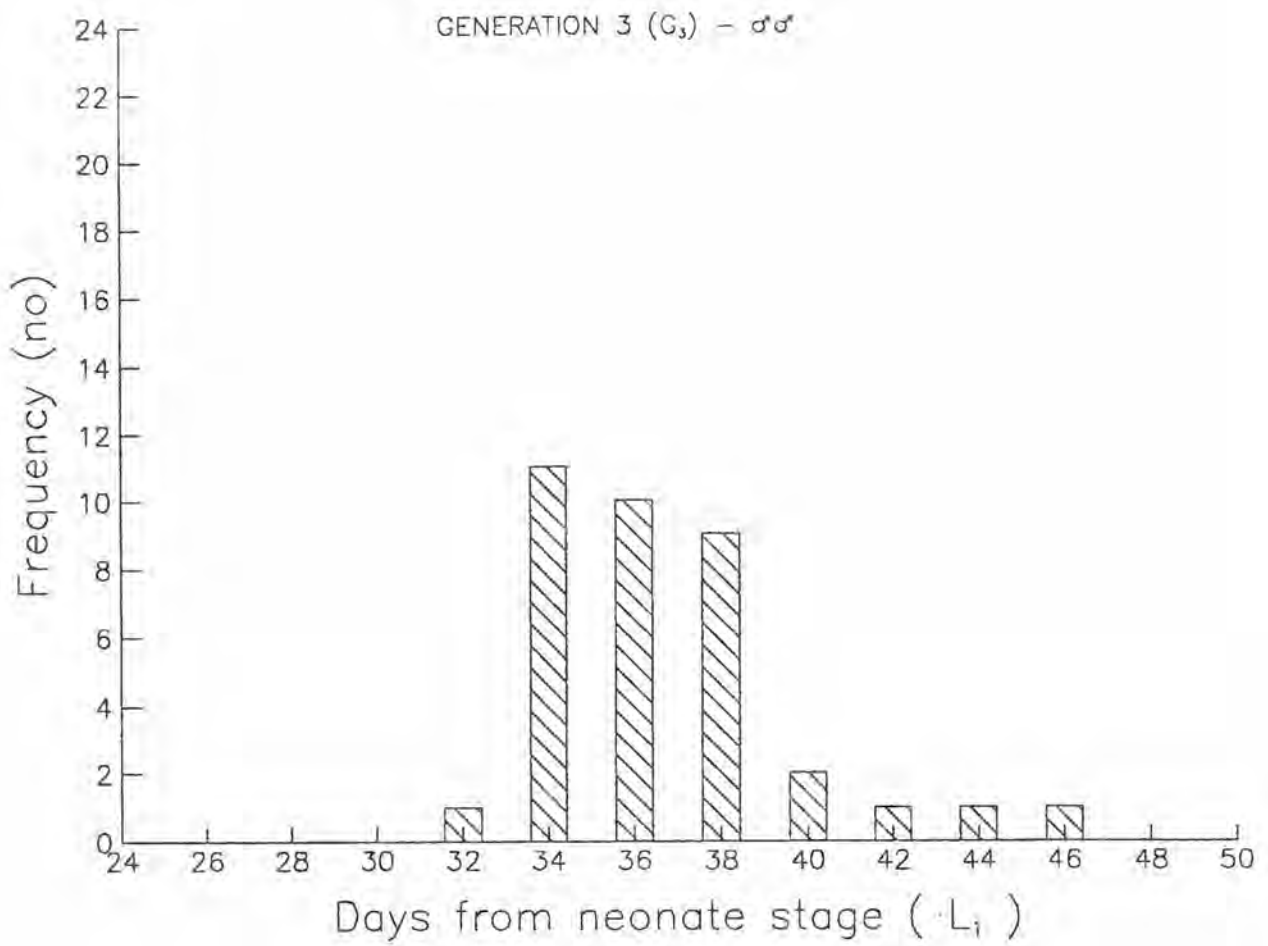
GENERATION 3 (G₃) – ♂♂

GENERATION 3 (G₃) – ♀♀

Figure 7.5 Distribution of pupation times over three

generations of assortative selection for

light pupal weight.
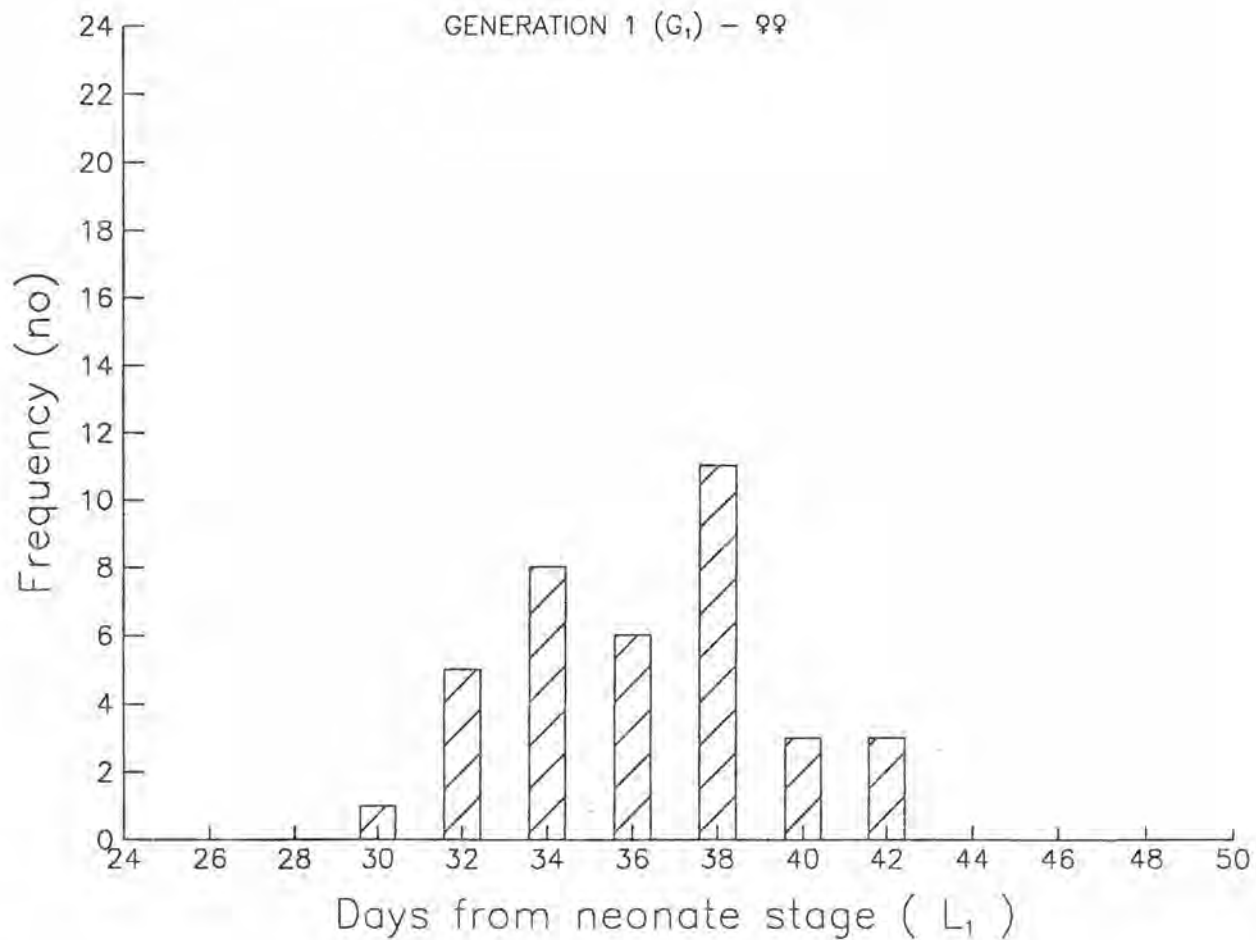
a) Generation 1

(n: male=40 female=39)
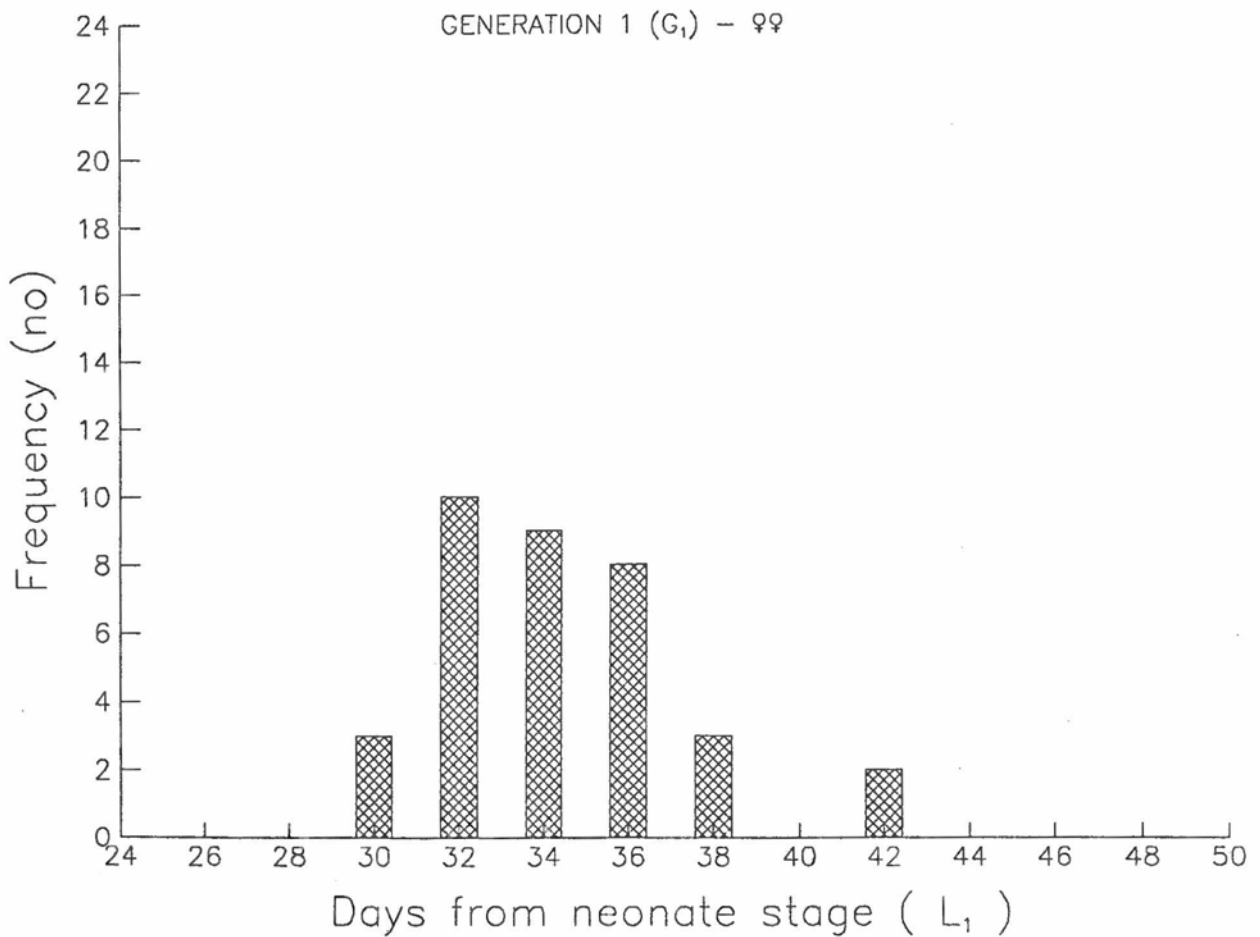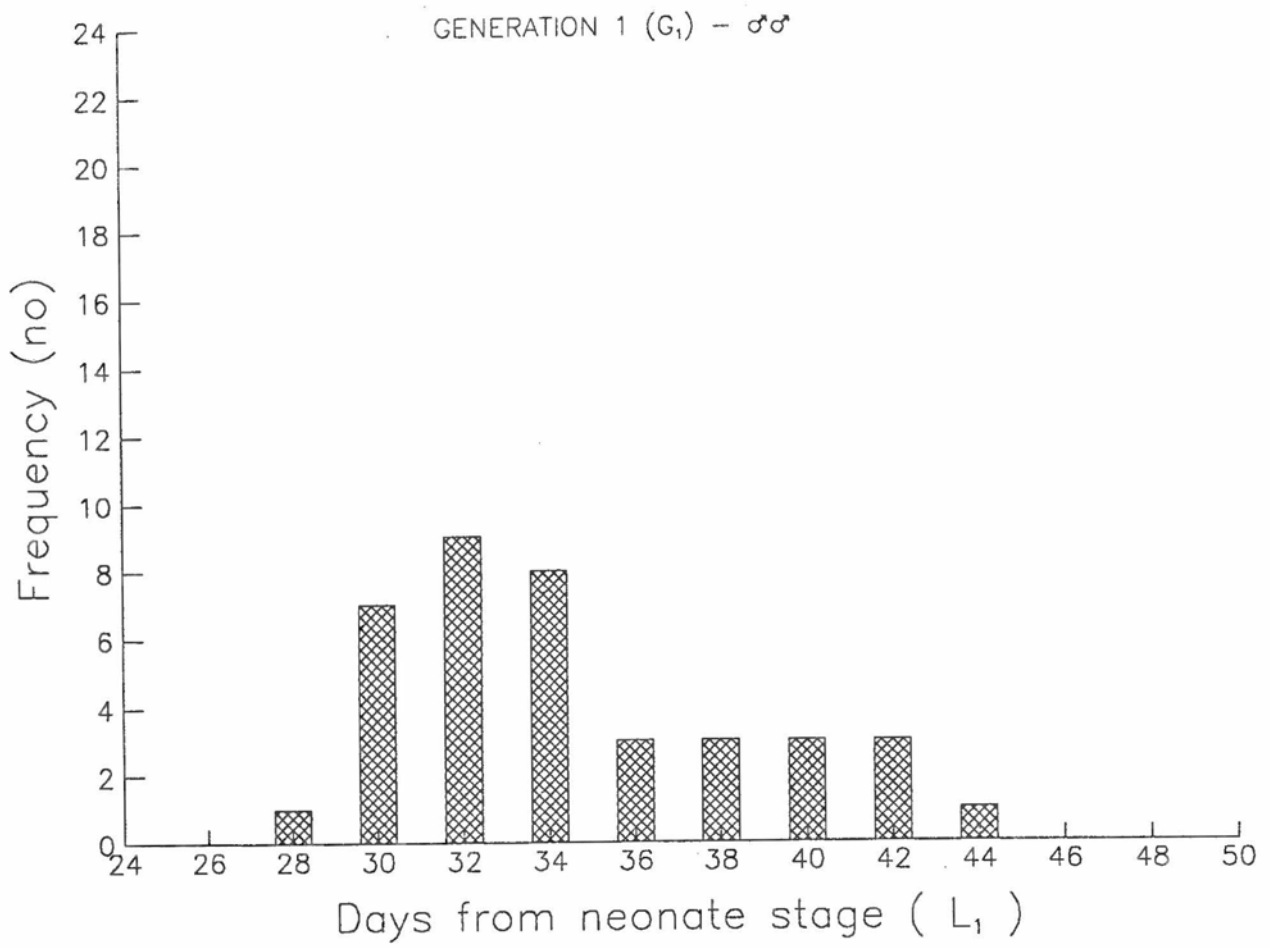
Figure 7.5 Distribution of pupation times over three

generations of assortative selection for

light pupal weight.

b) Generation 2

(n: male=38 female=35)
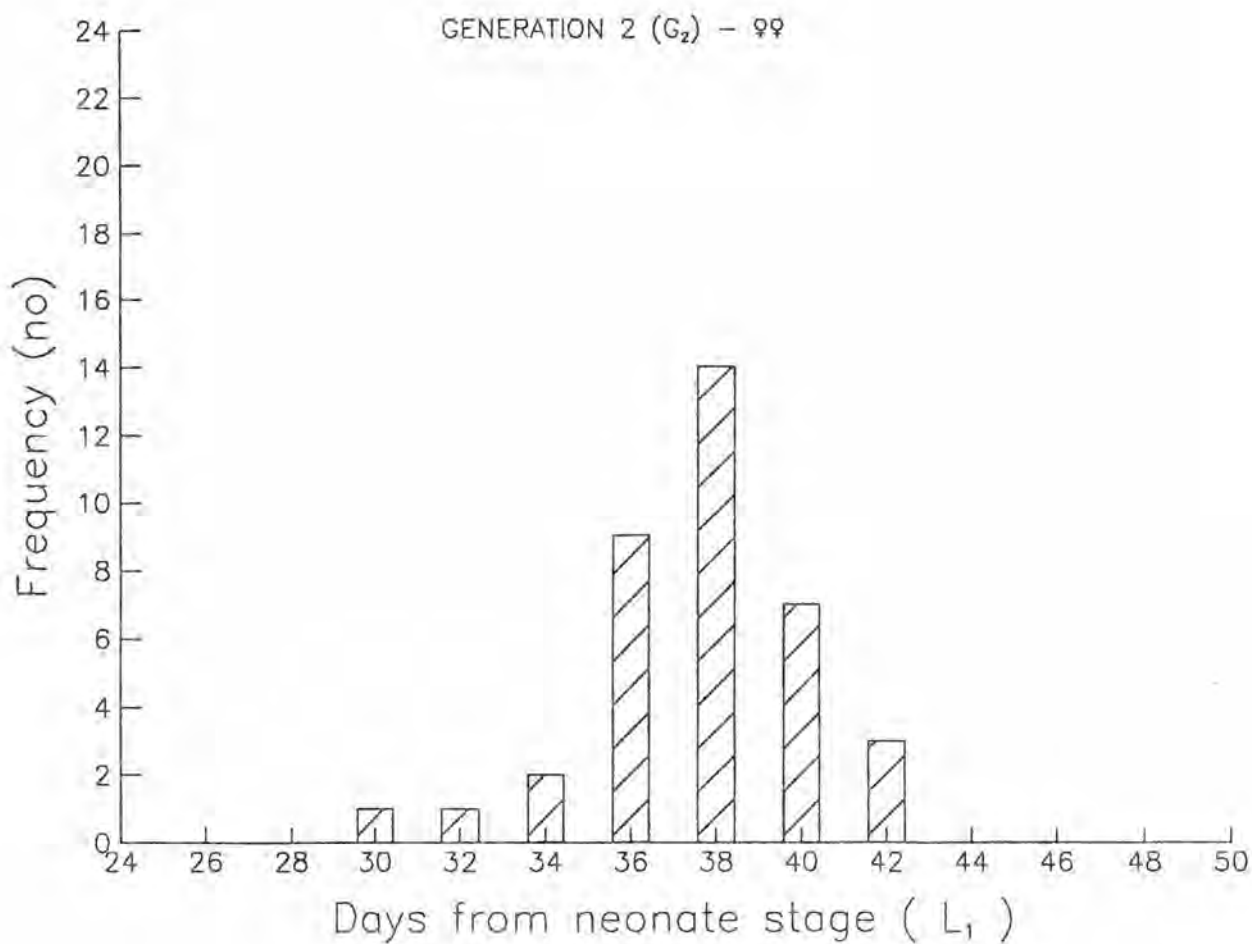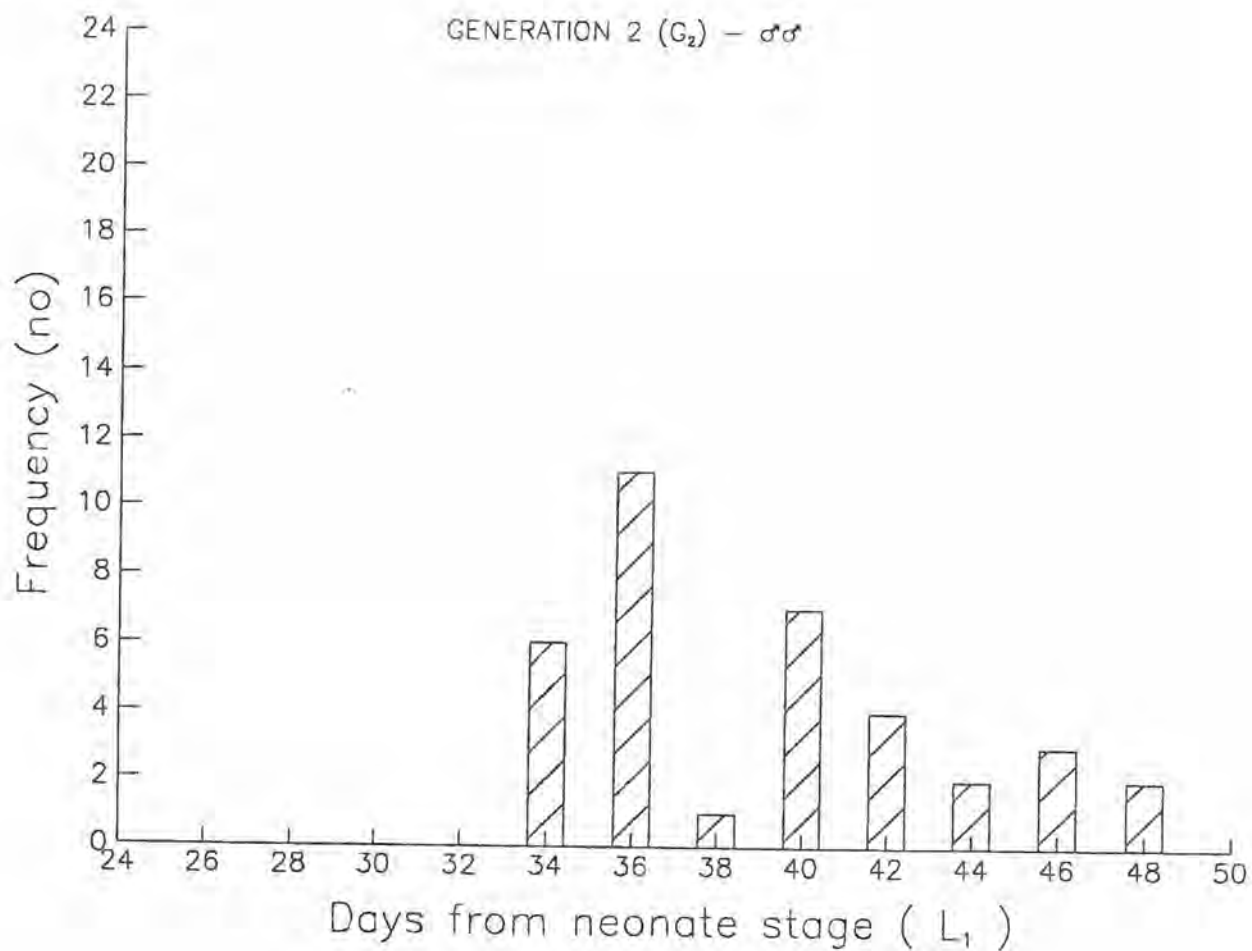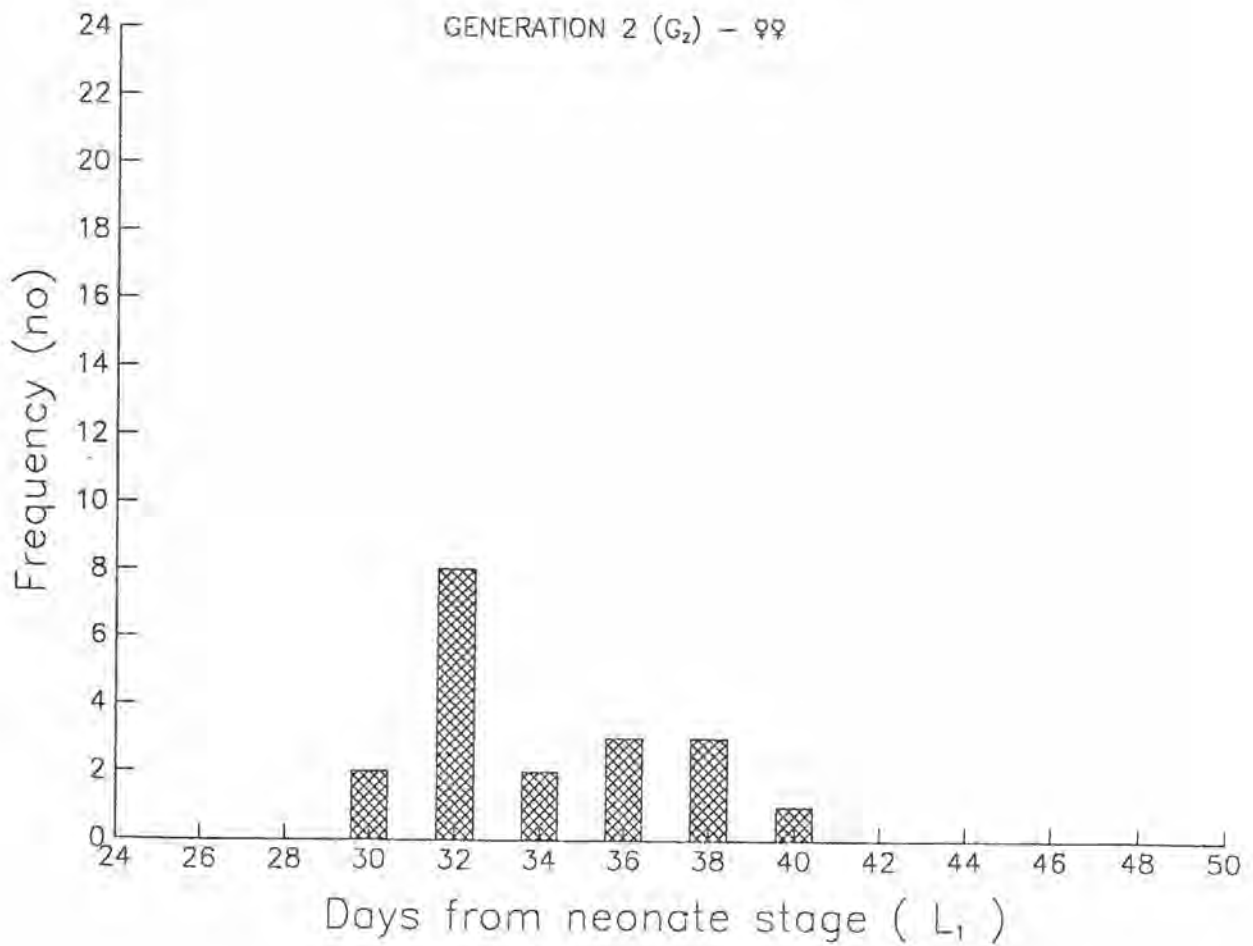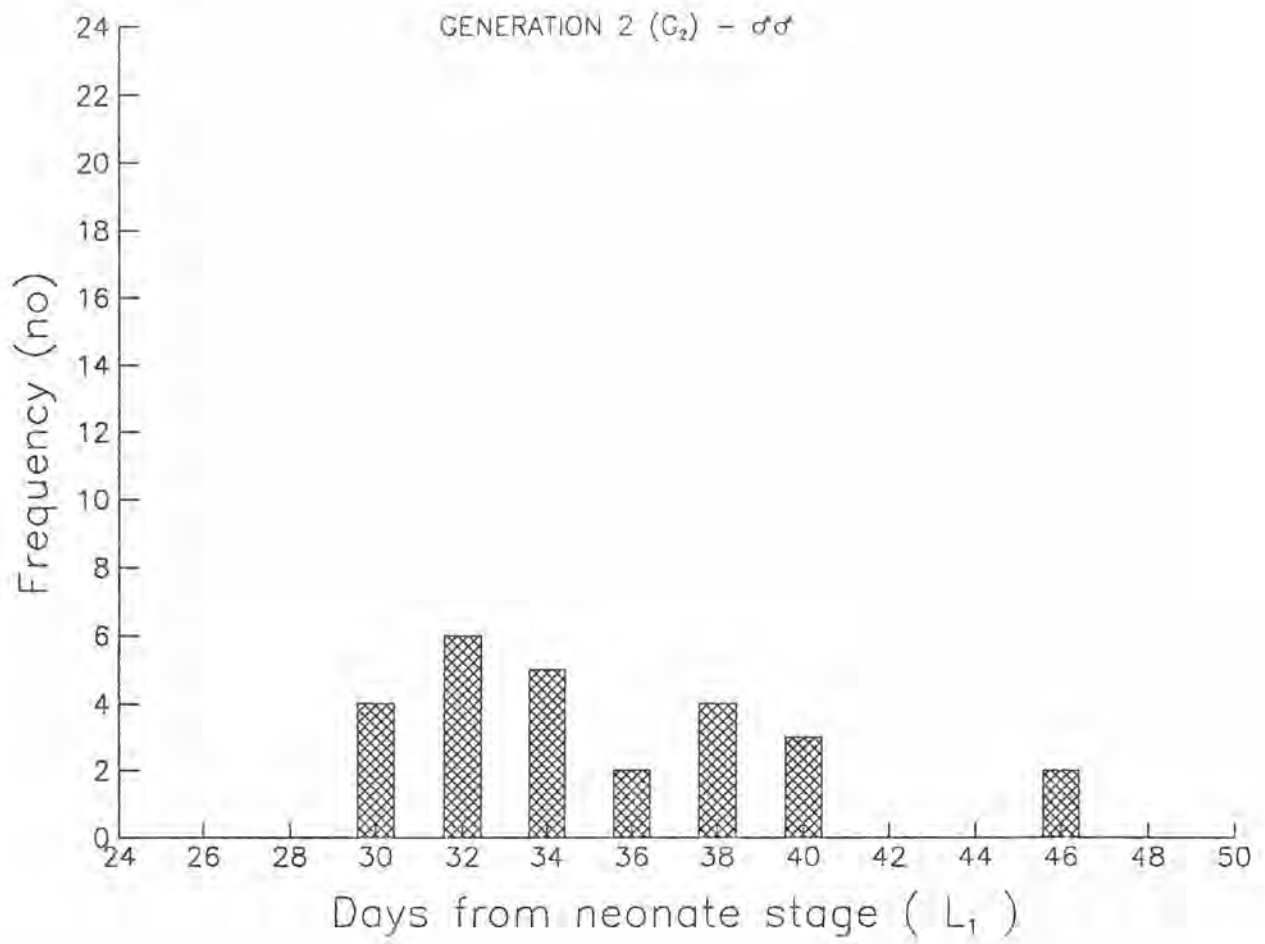
GENERATION 2 (G$_2$) – ♂♂
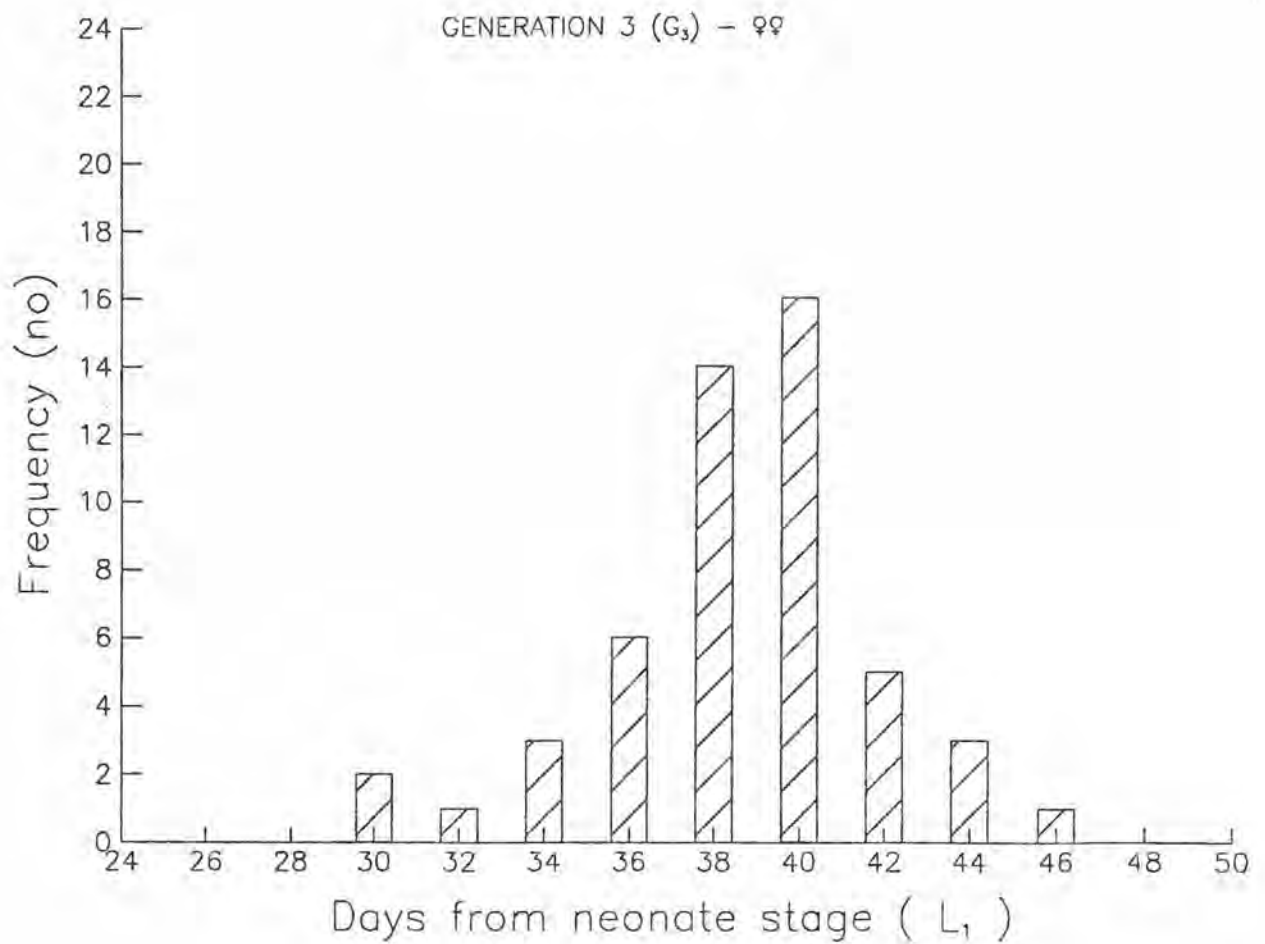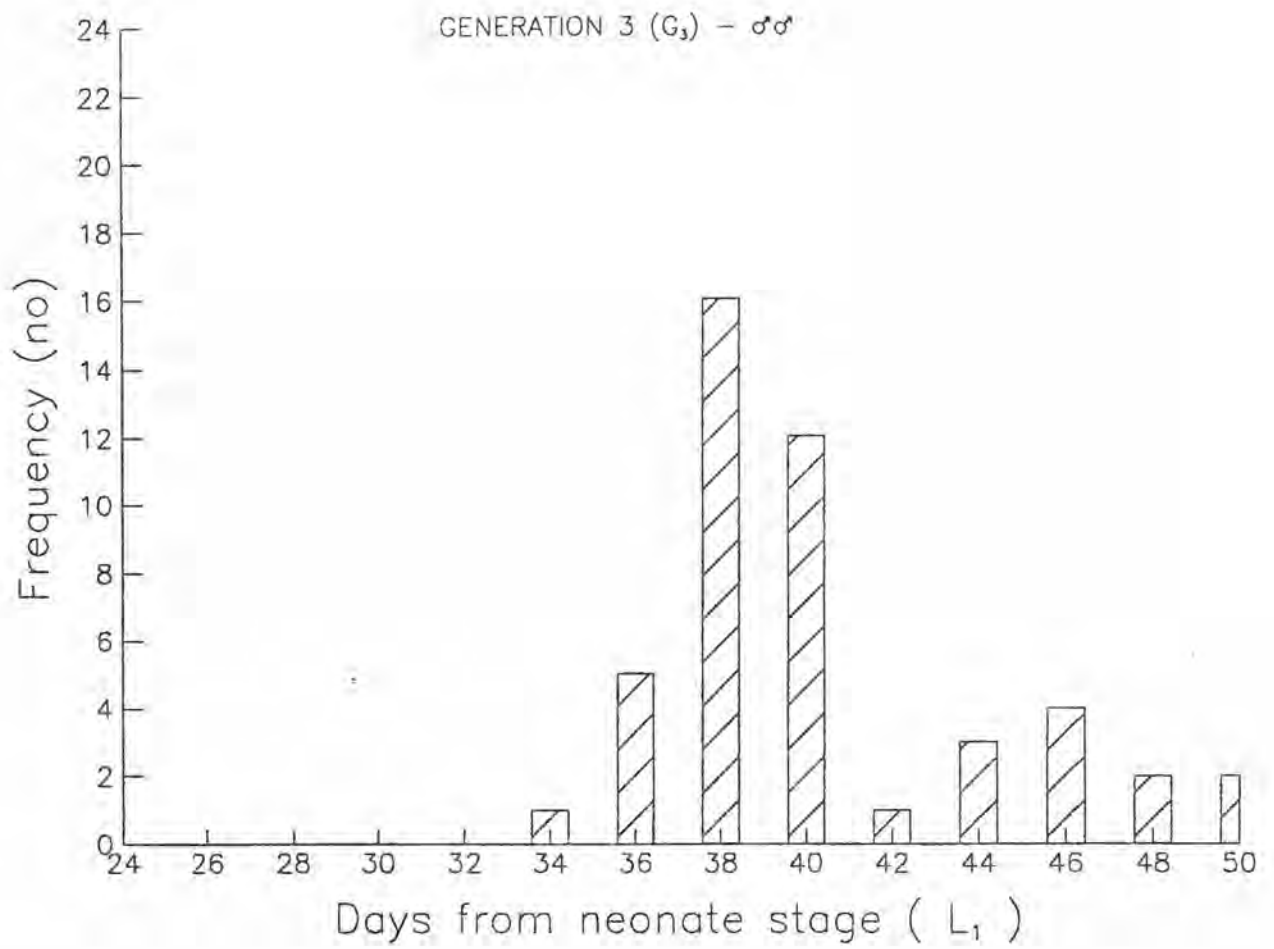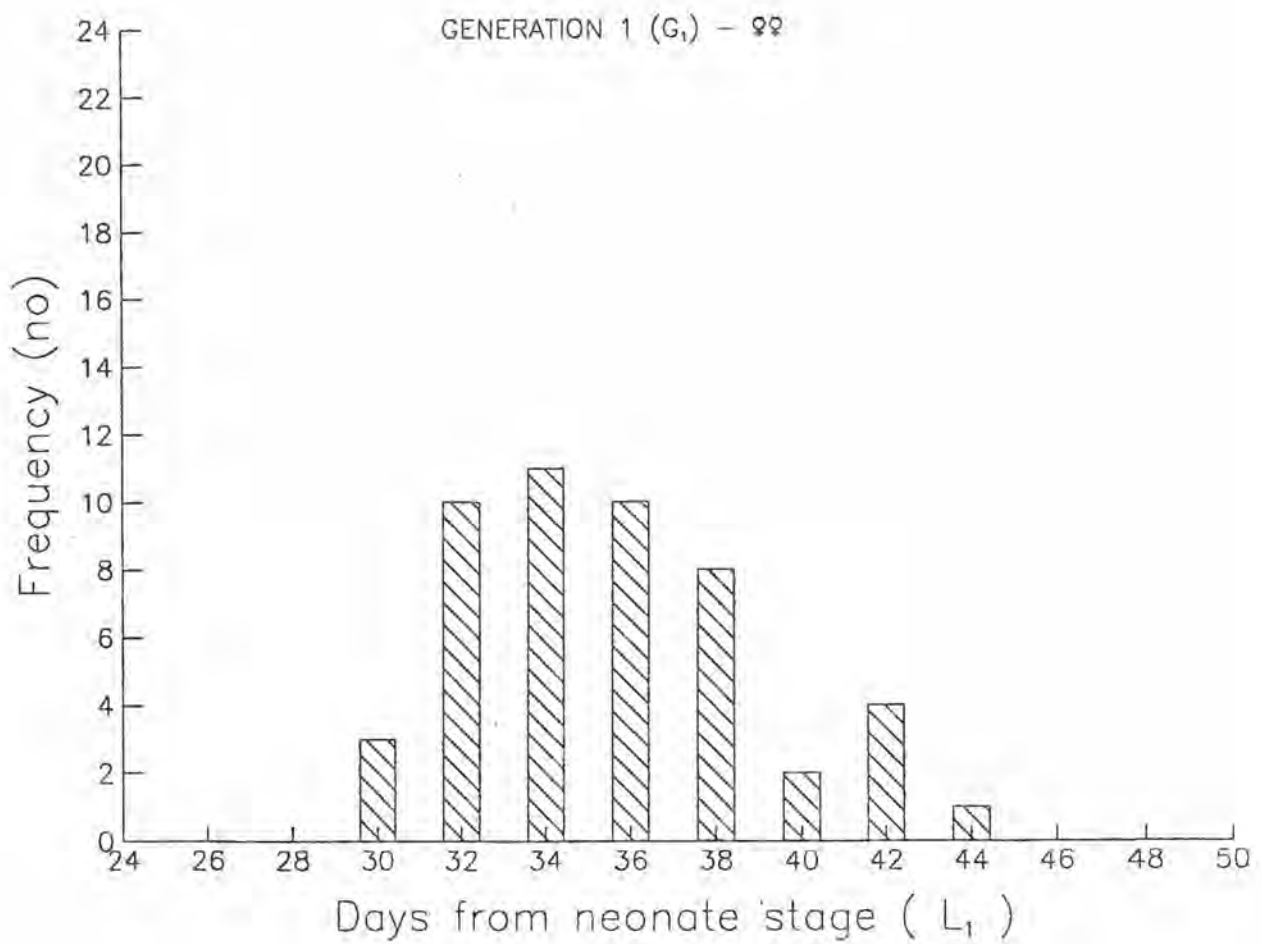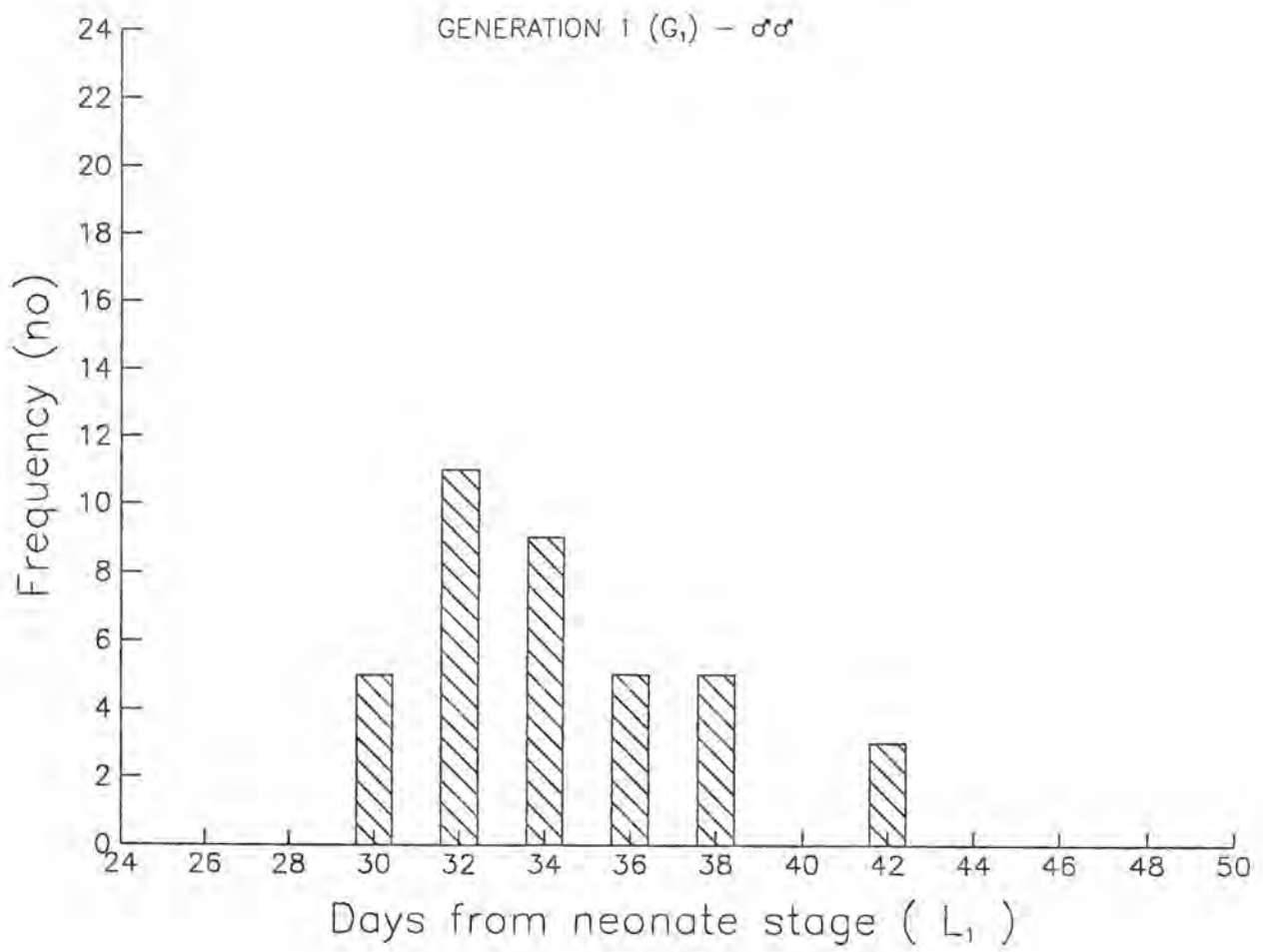
GENERATION 2 (G$_2$) – ♀♀

Figure 7.5 Distribution of pupation times over three

generations of assortative selection for

light pupal weight.

c) Generation 3

(n: male=23 female=19)

GENERATION 3 (G₃) – ♂♂

GENERATION 3 (G₃) – ♀♀
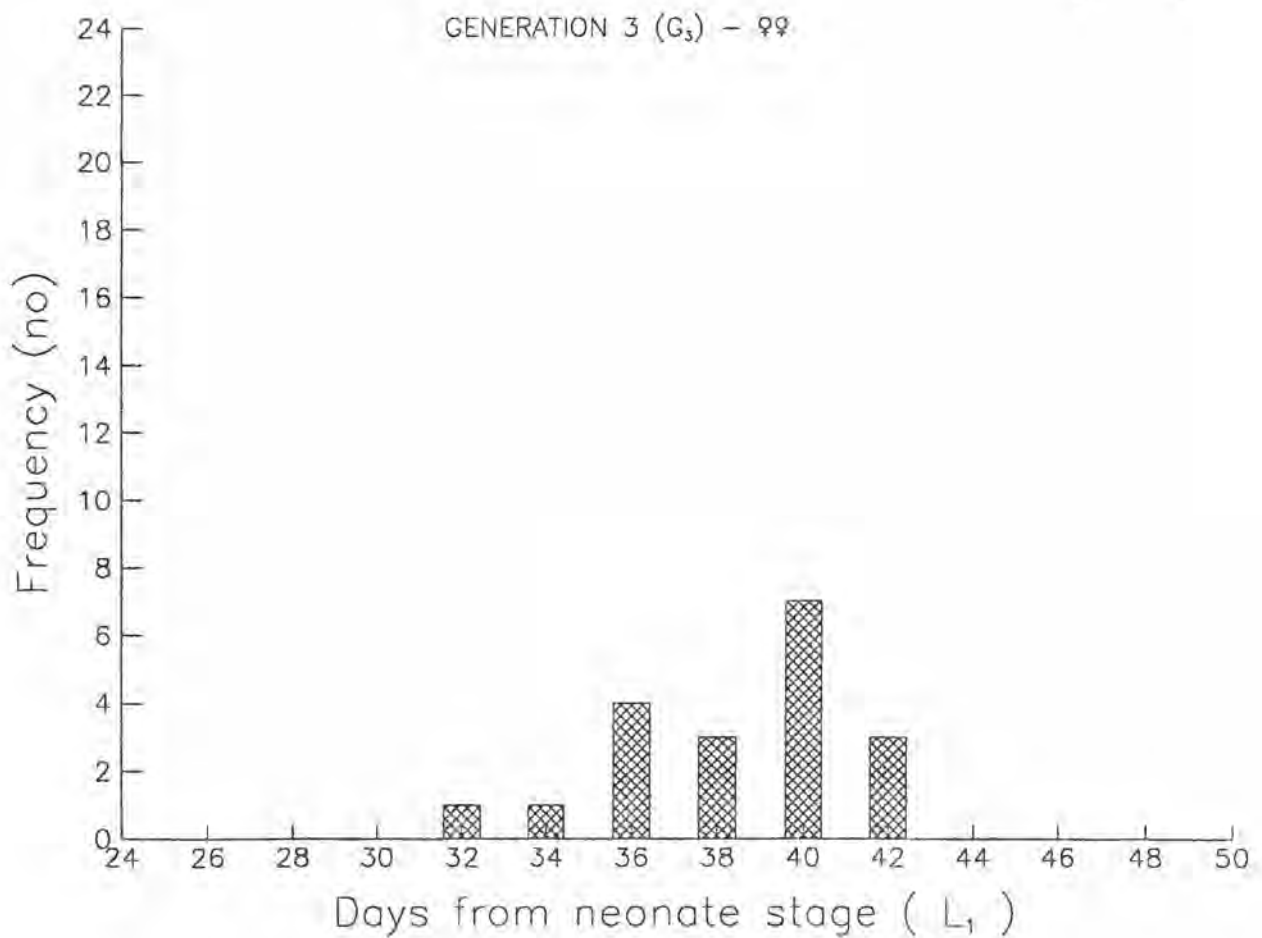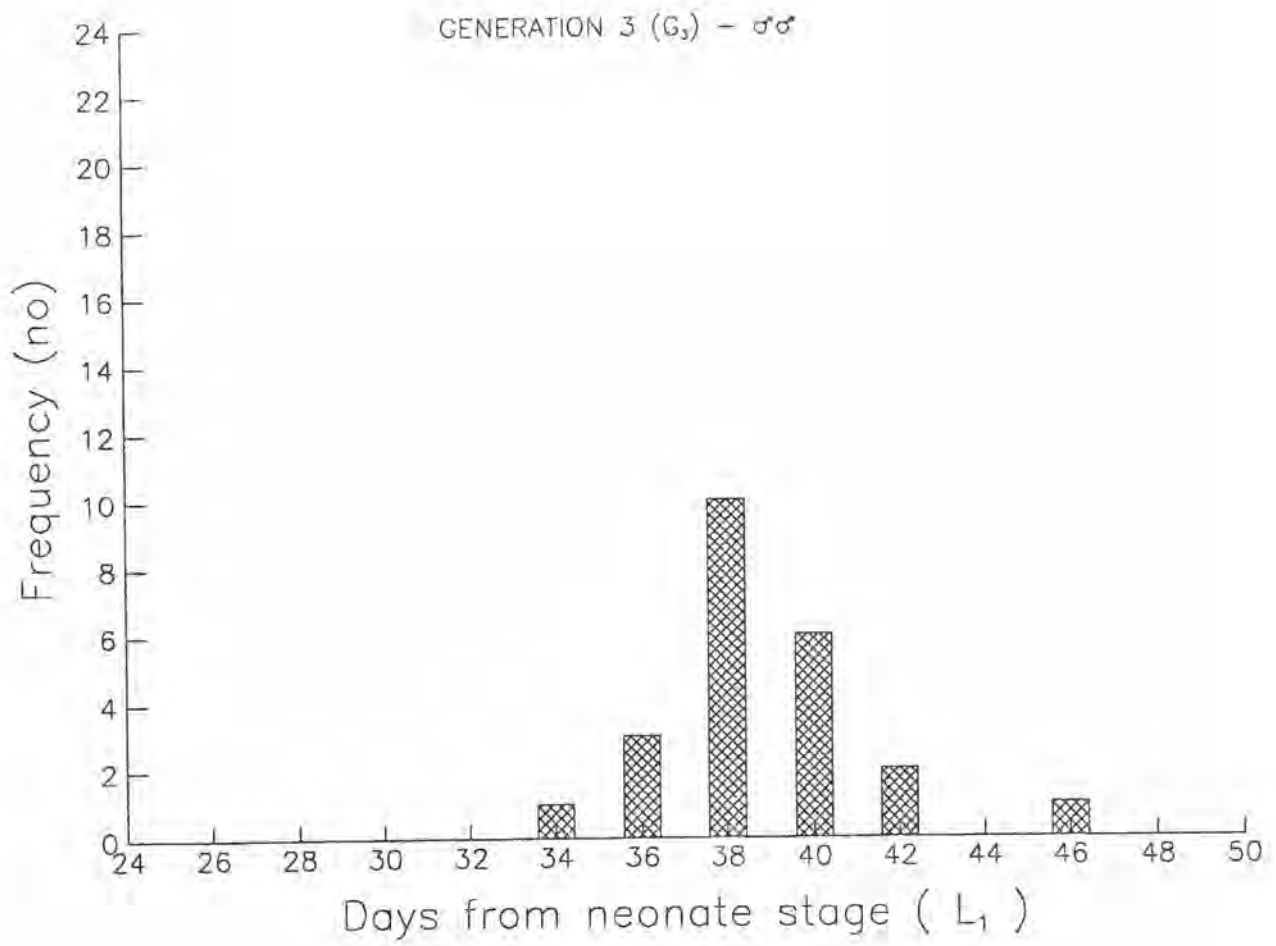
CHAPTER / 8

GENERAL CONCLUSION

This thesis departs from the popular view of the quality of insect populations reared in captivity as dependent only on their ultimate use (Huettel, 1976; Bush, 1979; Boller, 1979; Chambers, 1980; Moore *et al.* 1985). The thesis regards quality in terms of the success in survival and colonising ability (reproductive fitness). The laboratory environment is regarded as an ecological habitat (albeit artificial) that insects must colonise effectively in order to be able to survive and reproduce. Differences in the biological performance of wild and artificially reared insects are regarded to be due to different effects and demands influencing the insect populations in the two habitats (see chapter 1).

The thesis set out to examine the effect of artificial colonisation on insect quality; how body size (weight) is determined and how it relates to reproductive performance under different conditions. The results of the study can be summarised as follows:-

1) In 12 generations of successive rearing of *C. jactatana* no changes occurred that can be referred to as adaptions (Darwinian). The overriding requirement in the artificial rearing

of insects is to ensure that there is no difference in terms of performance and overall quality between the laboratory and the wild population. That strategy can be achieved by conserving the genetic variability of a colonised population. Artificial colonisation is successful within a limited range of environmental factors. Within the limits is a narrower preferred range of colonisation. Colonisation ranges should be as wide as possible to 'capture' much of the insect population's genetic variability. During laboratory colonisation selection, acclimatisation and domestication occur and may lead to changes in the biological performance of the laboratory population. The amount of change will depend on the resilience of the laboratory population to the artificial habitat brought about by the inherent variability.

2) Final instar larvae of *C. jactatana* have a critical weight (L_{CW}) which acts as threshold regulating pupal and adult size. Within a group of equal sized insects, larvae that pupate at the L_{CW} form pupae of the smallest size (pupal critical weight) and adults of the smallest size (adult critical weight). There was a proportionate decrease in weight from the maximum weight a larva attains in the final instar (L_{MW}) to pupa (constant D_P) and to adult (constant D_A). Size (weight) of pupa and adult depend on the level of the L_{MW} which in turn depends on the duration and quantity of feeding during the latent feeding period. There was a direct relationship between pupal size, adult size and reproductive

performance.

3) Diet quality had no effect on the L_{CW}, D_P or D_A though it affected L_{MW} and therefore pupal size, adult size, and reproductive performance of adults.

4) There was an inverse relationship between temperature and size. Decrease in temperature within a limited range that did not interfere with body metabolism increased pupal size, adult size, and the reproductive performance. Temperature, however, had no effect on the L_{CW}, the D_P or D_A.

5) Photoperiod did not affect the L_{CW}, pupal and adult weight although an increase in photophase prolonged the duration of the final instar. Pupal and adult weight, and reproductive performance increased in the thermophotoperiod where the thermophase coincided with the photophase.

6) Positive assortative selections for i) fast rate of development and ii) high pupal weight over three generations did not affect the reproductive performance. Selection for i) slow development and ii) low pupal weight significantly decreased pupal weight and reproductive performance. The L_{CW} was not affected by any of the selection procedures.

The use of an L_{CW} to define pupal and adult quality indices is useful in testing and predicting the performance of laboratory reared insects. The indices can also be used to compare the biological performance of similar species reared in different laboratories.

The major conclusion of this study is therefore, that because the L_{CW} was not affected by generations of successive rearing, assortative selection, diet quality, temperature or photoperiod it seems that the larval critical weight (L_{CW}) in *C. jactatana* is a mechanical trigger for pupation (possibly mediated through stretch receptors). Understanding the performance of artificially colonised insects is logical when the laboratory is regarded as an ecological habitat that the insect population must survive in and colonise. This approach is important as it brings a holistic functional perspective to the question of overall quality. The approach also allows the results of specific tests of overall quality to be interpreted as due to different effects and demands placed by the two habitats (laboratory and wild) on insect populations. Quality of an insect should therefore relate to the biological performance more in terms of survival and colonising ability rather than solely on 'intended role' or 'fitness for use'.

APPENDIX A.1

STANDARD REARING PROCEDURE USED IN THE LABORATORY MANAGEMENT OF
C. jactatana

A.1.1 Adult management

a) *Single pair oviposition unit* (open-ended perspex tubing,

150mm long and 30mm internal diameter, Singh *et al*. 1985).

*Cut 150x110mm polythene sheet filling the outside of the

perspex tube.

*Score several criss cross grooves on the polythene sheet

using the pointed ends of a pair of scissors.

*Insert the sheet into the tube to form an internal sleeve.

*Seal one end tightly with an absorbent cotton wool plug.

*Place one pair of adults into the tube.

*Seal the other end of the tube with a tight absorbent

cotton wool plug moistened in 10% honey solution.

*Prepare 20 tubes likewise.

*Label the tubes accurately and appropriately.

*Place the oviposition unit at the appropriate

environmental condition (Table A.3).

*Change moistened cotton wool every 2-3 days to avoid

dehydration and development of mould.

b) *Group oviposition unit* (transparent triseal polythene bag

250 X 375mm, Singh *et al.* 1985).

*Assemble feeding pottle with honey solution, plastic

tubing and dental roll wick.

*Make 6 holes evenly spaced on the polythene

bag using a 2mm piece of wire.

*Label the bag accurately and appropriately.

*Plug ca. 20mm hole at the bottom end of the bag.

*Place the galvanised wire in the triseal polythene bag.

*Seal the open end of the bag with a rubber band.

*Turn the bag over and place the plastic tubing and the

wick the of feeding pottle through the hole in the bag.

*Set 12 oviposition units per week.

*Place the oviposition unit at the appropriate

temperature (Table A.3).

A.1.2 Egg management

a) *From single pair oviposition unit.*

*Prepare a new oviposition unit.

*Transfer moths from the old unit to the new one.

*Remove the polythene sleeve with egg batches from the

unit.

*Carefully cut around individual egg batches using a pair

of scissors.

*Set up single egg batch incubation tubes (45 X 15mm).

*Place a single egg batch (ca. 30 eggs) face up in the
tube using soft-nosed forceps.

*Label the incubation tube accurately and appropriately.

*Incubate the tube at the appropriate temperature (Table
A.3).

*Collect eggs weekly until the the female is dead.

b) *From group oviposition unit.*

*Prepare a new oviposition unit.

*Remove the rubber band from the old unit containing egg
batches and carefully transfer moths into the new unit by
a sudden shake.

*Cut open the bag with eggs.

*Cut around individual egg batches carefully using a pair
of scissors.

*Set up egg incubation/storage units (plastic Chinese
containers 105mm diameter X 100mm high)

*Place the egg batches face up in the Chinese containers
using a soft-nosed forceps (ca. 500 eggs per unit).

*Label the container accurately and appropriately.

*Incubate/store at the appropriate environmental condition
(Table A.3).

*Collect eggs twice (weekly) before discarding the
adults.

A.1.3 Larval management

a) *Single rearing units* (polystyrene test tubes, 75 X 12mm)

*Condition diet by allowing test tube to stand at rearing
temperature overnight.

*Sterilise brush heads in 5% sodium hypochlorite solution
for 15 minutes. Rinse well in distilled water and dry
with a soft tissue paper. Place brushes under u.v. light
for 15 mins.

*Transfer 1 neonate larva per tube carefully onto diet
using a camel-hair brush.

*Plug test tube tightly with cotton wool.

*Place test tube upright in test-tube rack or plastic
holding container.

*Label the rack accurately and appropriately.

*Place the rearing units at the appropriate
environmental condition (Table A.3).

b) *Group rearing units* (transparent plastic container
(190 X 120 X 65mm, Clare *et al.* 1987).

*Condition the diet in the group rearing units
by placing them at room temperature overnight before

inoculation.

*Sterilize eggs in 2.5% formaldehyde solution for 15 minutes. Rinse the eggs in three changes of distilled water.

*Place a minimum of 10 egg batches (containing a total of ca. 200 eggs) of 'blackhead' stage on top of the filter paper on the light diffuser panel.

*Place a sheet of synthetic porous material (Tyvec^T) on top of the plastic container.

*Press the lid (ca. 11x20mm holes) firmly into place sealing the container.

*Label the units accurately and appropriately.

*Inoculate 5 group rearing units per week.

*Place the group rearing units at the appropriate environmental condition

(Table A.3).

*Remove the filter paper and eggs that are not hatched after one week.

A.1.4 Pupal management

a) *From single rearing units*

*Remove diet or frass necessary to uncover the pupa with a wire hook.

*Carefully dislodge the pupa from any webbing or diet and

extract from the tube using a wire hook.

*Sex the pupae (male has 5, smaller, compact, ventral abdominal segments; female has 4, large, spaced ventral abdominal segments.

*Place the pupae in pupal incubation tubes (13 X 90mm).

*Label the pupal incubation tubes accurately and appropriately.

*Place in the appropriate environmental condition (Table A.3).

b) *From group rearing units*

*Prepare a strip of polythene (41 microns thick, 1500 X 30mm).

*Roll the strip into a 20-25mm wide roll and hold the roll in place by a paper clip.

*Introduce two rolls into each container, placing the rolls upright between the light diffuser panel and the lid 2-3 days before the pre-pupal stage (30 days from inoculation of neonates at 18°C).

*Open the roll to harvest pupae.

*Sex the pupae.

*Place 30 pupae per plastic Chinese container.

*Label the containers accurately and appropriately.

*Incubate/store the pupae in the appropriate environmental condition.

APPENDIX A.2

REGULAR QUALITY ASSESSMENT TESTS FOR THE LABORATORY COLONY OF *C. jactatana*

The following are a series of standardized tests that the laboratory colony of *C. jactatana* had to undergo in each generation in order to maintain the level of quality and ascertain that insects produced gave reproducible performance.

A.2.1 Quality in adults

a) *Fecundity and fertility* of mated adult females was a reliable indicator of the adequacy of larval and adult diet, oviposition environment (e.g. humidity level) and genetic condition of the colony (n=20).

b) *Adult Mortality/longevity* served as an indicator of the general health of the colony. Diseased adults and inappropriate oviposition environment levels were detected by reduced longevity (n=20).

c) *Adult Malformation* indicated nutritional inadequacy of the larval diet and problems in pupal management (n=20).

d) *Mating success*: Dissection of females to

detect the presence or abscence of spermatophores

indicated the responsive behaviour of males to

attractive females. Number of spermatophores (single

or in multiples) indicated number of

successful matings (n=20).

e) *Female attractancy* was tested by tethering females

overnight in field cages. A test of the cumulative

attractive factors that make females functional in a

semi-wild situation (n=20).

f) *Pheromone production*: Quantitative estimation of one

attractive pheromone component

((Z)-tetradecenyl acetate) of 2-5 day old virgin

females by capillary GLC analysis using a 50m BP20

capillary column indicated changes that occured in the

species mate recognition system (SMRS) over successive

generations of rearing (n=50).

A.2.2 Quality in Eggs

a) *Egg hatch*: The percentage of the total eggs laid that

hatched indicated success of mating, dietary adequacy,

and the suitability of the egg incubation environment. Egg batches (ca. 30 eggs each) of each generation were monitored at $18 \pm 1°C$ (n=20).

A.2.3 Quality in larvae

a) *Head capsule measurements* was used to monitor larval size in each generation.

b) *Instar/stadia development* was used to monitor the number of instars and their duration in each generation.

c) *Larval abnormalities*: A record of the number of larval malformations obtained in each generation was kept.

e) *LD-50 susceptibility* of neonates using *Bacillus thuringiensis* (BT) endotoxin was used to monitor low level trans-ovarial microbial infections. Infected neonates had higher susceptibility levels to BT.

f) *Smear tests* were used to screen larvae suspected to have microsporidian infections.

Quality assessment of larvae was conducted on 100 larvae selected at random in each generation.

A.2.4 Quality in Pupae

a) *Pupal weight* variation over each generation.

b) *Sex ratio* for extreme deviation from a 1:1 ratio.

c) *Pupal abnormalities* : Record of the number of deformed
   pupae in each generation.

d) *% Non eclosion* Record of the number of pupae that are
   unsuccessful in eclosion.

e) *Free amino acid (FAA) profiles* of fresh pupae of both
   sexes using an HPLC amino acid analyser was used as a
   test for the stability of the internal biochemistry over
   successive generations.

The quality assessment of pupae was performed on 100
pupae of each generation.

A.2.5 Quality in the Rearing Process

a) *Inspection* at strategic locations of the rearing flow
   process (e.g. diet dispenser, autoclave, temperature
   and light controls etc.).

b) **Quality** of stored products (e.g. inspection of dietary
   ingredients and stored diet etc.).

The assessment tests listed above were adequate in
regulating the quality of the laboratory population for
experiments described in the preceding chapters. To ensure that

overall quality is maintained in laboratory reared insects used in field release situations, several other tests need to be considered. Adult flight and orientation, adult sexual activity (mating propensity, frequency and compatibility), sexual physiology (sperm/egg production, sexual maturation) as well as the genetics of inbreeding.

Table A.1 Head capsule (Width) measurements (mm) of larval
instars of *C. jactatana* reared on GPD artificial
diet at 18 ±1°C, 75 ±5%RH, LD 18:6

Instar	Sex	Mean «±»95% C.I.	Range
1	♂	0.23 0.22«±»0.24	0.20 - 0.28
	♀	0.23 0.22«±»0.24	0.20 - 0.28
2	♂	0.35 0.34«±»0.36	0.30 - 0.40
	♀	0.35 0.34«±»0.36	0.30 - 0.40
3	♂	0.53 0.52«±»0.54	0.50 - 0.60
	♀	0.53 0.52«±»0.55	0.38 - 0.63
4	♂	0.84 0.83«±»0.85	0.75 - 0.90
	♀	0.84 0.83«±»0.85	0.70 - 0.93
5	♂	1.19✦✦✦ 1.17«±»1.21	1.10 - 1.30
	♀	1.24✦✦✦ 1.23«±»1.25	1.13 - 1.38

n   ♂ = 30   ♀ = 52
✦✦✦   values significant at p< 0.001 (paired sample t-Test)

Table A.2 Containers used in the rearing management of C. jactatana

Stage	Purpose	Container	Specification
	Holding	Plastic Chinese container	105mm dia.X 100mm high
Adults	Single pair mating	Perspex tubes + Polythene sleeves	30mm dia.X 150mm long 150mm X 110mm
	Group mating	Transparent triseal polythene bag + Plastic tray + Clear plastic pottle + Plastic tubing + Cotton dental rolls + Galvanised wire frame	250mm X 375mm 340mm X 455mm 60mm dia. X 50mm high 25mm dia X 35mm high 75mm
Eggs	Single batch incubation	Perspex tubes	45mm X 15mm
	500 batches incubation	Plastic Chinese container	105mm dia X 100mm high
Larvae	Single rearing	Polystyrene test tubes	75mm X 12mm
	Group rearing	Transparent plastic container + Light diffuser panel + Porous material	190mm X 120 X 65mm 180mm X 115 X 14mm 230mm X 160mm
Pupae	Single rearing	Perspex tubes	13mm dia. X 90mm high
	Group rearing	Plastic Chinese container + Strip of polythene roll	105mm dia X 100mm high 1500mm X 30mm

Table A.3 Environmental conditions used in the rearing management
        of *C. jactatana*

Stage	Purpose	Temperature (±1ºC)	Photoperiod (L:D)	Humidity (±5%RH)
Adults	Holding	10	16:8	40
	Rearing	15	16:8	75
Eggs	Holding	12	0:24	70
	Rearing	18	18:6	75
Larvae	Rearing	18	18:6	75
Pupae	Holding	10	0:24	40
	Rearing	18	18:6	75

Light type: 6 overhead fluorescent cool white tubes, 65W/33RS
            covered with transparent multifaceted perspex.

Light intensity: 875 lax.

Airflow system : 0.559m³/sec, fresh air mixed at 0.10m3/sec.

Table A.4 Composition of dietary concentrations of a general
purpose diet (GPD, Singh 1983) evaluated for the
rearing of *C. jactatana*

Ingredient	% concentration of GPD	
	100%	25%
Dry mix*	203.3g	50.9g
Distilled water	815.0ml	815.0ml
Potassium hydroxide	5.0ml	5.0ml
Agar	−	18.5g
Mould inhibitor**	15.0ml	15.0ml
Vanderzant vitamin mixture	20.0g	5.0g
Sucrose	30.0g	7.5g
Glucose	5.0g	1.3g
Streptomycin	150.0mg	150.0mg
Penicillin	150.0mg	150.0mg

*Composition of dry mix/100g

Agar	12.5g
Casein	17.5g
Cellulose powder	50.0g
Wessons salt mix	5.0g
Wheat germ	15.0g
Cholesterol	0.25g
Linoleic acid	1.25ml

**Composition of mould inhibitor

Methyl p-hydroxybenzoate	15.0g
Sorbic acid	20.0g
Ethanol (95%)	175.0ml

Table A.5 Composition of Sheep nut - bean based diet (SBD, modified
after Shorey and Hale, 1965 and Thomas, 1968   )

Ingredient	Quantity
Sheep nut powder*	100g
Lima bean powder	75g
Lucerne leaf powder	25g
Agar	10g
Brewers yeast	30g
Citric acid	9g
Octave W.P.**	30mg
Sorbic acid	1.6g
Nipagin	1.4g
Distilled water	720.0ml

*Composition of sheep nuts

Barley meal	Lime
Linseed meal	Salt
Pea meal	Urea
Lucerne meal	Potassium iodide
Bran	Copper sulphate
Pollard	Cobalt sulphate

**Fungicide containing prochloraz at 450/kg

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