DOCTOR OF PHILOSOPHY

The physiological ecology of the phase polymorphism in the African armyworm moth, Spodoptera exempta (Walker) (Lepidoptera: Noctuidae).

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Award date:
1995

Awarding institution:
Bangor University

THE PHYSIOLOGICAL ECOLOGY OF THE PHASE POLYPHENISM IN THE AFRICAN ARMYWORM MOTH, *SPODOPTERA EXEMPTA* (WALKER) (LEPIDOPTERA: NOCTUIDAE)

A thesis submitted

by

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for

the degree of Doctor of Philosophy

of

the University of Wales

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April, 1995

"...the biological background of phase changes is anything but simple and clarity could be achieved only by deliberately ignoring its complexity" .

(Uvarov, 1966).

Summary

The effects of larval phase polyphenism on adult reproductive biology, and larval nutritional and thermal ecology of the African armyworm moth, *Spodoptera exempta* (Walker) were investigated.

Differences between moths which were reared from *solitaria* and *gregaria* larvae were obtained in fecundity (weight-adjusted) and pre-oviposition period. Number of egg batches, oviposition period and longevity of female moths were influenced by adult diet and not by larval phase. Phase effects on fecundity and pre-oviposition period were removed by rearing larvae as *solitaria* for three generations.

Larval food consumption and utilization indices were affected by phase and instar; the direction of differences depended on whether *solitaria* larvae were first- or third-generation. There were no differences in growth rates between phases.

Weight-adjusted triglyceride contents of larvae were higher in the *gregaria* in two out of three trials. *Solitaria* larvae contained more body water. Rearing of larvae in different sizes of containers did not affect the triglyceride levels within phases, but affected water content in the *solitaria*.

At constant ambient temperatures of 17.5, 22.5 and 25°C, *gregaria* larvae developed faster than *solitaria*. At 30°C, larval period was shorter for the *solitaria* than the *gregaria*. The rate of development did not differ between phases at 35°C. *Gregaria* larvae lost their black pigment at 35°C and above. Anaesthetized black larvae attained higher rates of increase in temperature than larvae of lighter pigment when exposed to radiant energy. Loss of the black pigment at higher temperatures was accompanied by loss of ability of *gregaria* larvae to heat up faster under radiant energy. Studies on behaviour failed to demonstrate any preference of larvae in one phase for illuminated or shaded areas.

The implications of these findings for the life history of *S. exempta* and the problems involved in research on phase polyphenism are discussed.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following:

Dr A.G. Gatehouse for the guidance he provided as my supervisor.

Mr A.D. Davies and Mrs Susan Plumpton for technical assistance.

Dr E.M. Rollins (Southampton University), Mr Alwyn Owens (SEES, Bangor) and Mr Saeed Raisani (Reading University) for technical information on radiant energy.

Mr Edward Evans and staff of the SBS workshop, Bangor, for helping me construct pieces of equipment.

Mr Nigel Brown and staff of the botanic gardens, Treborth for growing the plants used in the experiments.

Dr J. Sook Chung and Dr John Blay for their assistance.

Dr K. Wilson (Cambridge University) and Dr A. Gunn (John Moores University) for sharing their experience with armyworms.

Dr C.J. Whittaker for statistical advice.

The Ministry of Agriculture, Ghana, for records on armyworm outbreaks and P.K. Kwapong who helped to obtain these records.

The University of Cape Coast (Ghana) for study leave and the Commonwealth Scholarship Commission in the United Kingdom for providing funds for this research.

Finally, Regina for all the sacrifices she made to support me all the way.

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CHAPTER ONE

GENERAL INTRODUCTION

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1.1 The African armyworm: general features and occurrence

The noctuid moth, *Spodoptera exempta* (Walker), is one of eight species in this genus that have been recorded in Africa and the near East. It occurs widely in tropical Africa, southwest Arabia, south-east Asia and Australia. The Americas appear to be free from this species, but there are records of its occurrence in Hawaii (Brown and Dewhurst, 1975; Odiyo, 1979; Haggis, 1986). Hill (1975) describes the moth as being night-flying and grey-brown, having pale hindwings with a conspicuous kidney-shaped whitish mark on the forewings and a wing span of about 28 mm. It is not clear which sex this description refers to as it makes no mention of the sexual dimorphism in appearance, the female moths being darker than the males.

The larva of *Spodoptera exempta* is commonly known as the African armyworm, a name that Brown (1962) considers most appropriate because this species is " best represented in Africa which appears to be its true home". The species is known by a variety of common names in different parts of its global range. These include 'mystery worm', 'army mystery worm', 'true mystery worm', 'South African mystery worm' (South Africa), 'swarming caterpillar' (Rhodesia, i.e. present Zambia or Zimbabwe), 'variegated armyworm', 'leaf-eating grass-worm' (Australia), 'grass armyworm', 'nut-grass armyworm' (Hawaii), 'yellow-striped army caterpillar' (Java), 'chenille légionnaire' (Madagascar) common armyworm, and 'day-feeding armyworm' (Brown, 1962; Brown and Dewhurst, (1975). These names and their countries of origin, indicate the wideness of the range of distribution of

the species, as well as aspects of its behaviour.

Outbreaks of the larvae occur sporadically, being most commonly reported on grassland and on grain crops, particularly maize, sorghum, millet, rice and wheat (Haggis, 1986), hence the 'grass' in some of the common names, such as 'grass armyworm' and 'nut grass armyworm'. These common names also reflect the oligophagous nature of the larvae which feed almost exclusively on Gramineae and Cyperaceae. According to Brown (1962), there is strong evidence that *S. exempta* is virtually confined to the Gramineae in spite of "inconsistent reports" of feeding on other plants. Brown (1962) also argues that since records on food plants are made almost entirely during outbreaks (when a species is most likely to turn to other plants because of possible exhaustion of preferred species), perhaps *S. exempta* is more closely restricted to Gramineae in non-outbreak periods than recorded food plants indicate. This argument assumes that the crowded larvae in outbreaks are characterised by a wider tolerance of plant species. The basis of the oligophagy is thought to be a combination of strong intolerance to chemical feeding inhibitors and a requirement for a well balanced complex of feeding stimulants (Ma, 1976; Ma and Kubo, 1977). The name "mystery worm" relates to the sudden, unexpected manner in which the caterpillars appear, often in large numbers, devour crops and pastures and, as abruptly, disappear.

The term "armyworm", derives from the ability of the larvae to 'march' in large numbers in search of new host plants after they have eaten out their immediate food supply. This habit is not peculiar to *S. exempta*, but occurs to varying degrees in several

other species both within and outside the genus *Spodoptera* (Brown, 1962). Therefore, the name armyworm may be applied to these species as well. According to Brown and Dewhurst (1975), the best known armyworms are in the genus *Spodoptera* and the 'marching' associated with the larvae of this species is probably not a direct response to the pressure of hunger, but rather to the high larval densities, which have the effect of a general, mutual stimulation to activity.

Virtually all the research work on this species has been done in eastern and southern Africa, where it has been a major agricultural problem. Haggis (1986) wrote that throughout the range of *S. exempta*, the extent and frequency of its attacks had increased markedly, particularly in the 10-15 years preceding her study. Since 1936, outbreaks had been reported each year in one part or another of the African continent. Only in three countries south of the Sahara (Benin, Equatorial Guinea and Central African Republic) were there no records of the existence of this species. Before 1970, few outbreaks were recorded in West Africa, but since then, there has been a marked increase in the number of countries attacked in a single season, and the reports from individual countries (Haggis, 1986).

Table 1 is based on unpublished data obtained from the Ministry of Agriculture in Ghana, which exemplify the trend of changes in the history of outbreaks in West Africa. These data show that after the first outbreak in 1937, it took thirteen years for the next to occur, while twenty-five years separated the third outbreak from the second. Between 1980 and 1992, an outbreak was recorded in each year (except 1990) in at least one locality. From 1987 to 1989, the

minimum number of regions (out of ten) in which outbreaks were recorded was five, while the maximum number of records in preceding years was three.

Lambert (1989) states that the seriousness of armyworm outbreaks in West Africa can be attributed largely to the absence of any kind of forecasting system and the inability to recognise armyworm stages before they have already caused damage. This has led the United Nations' Food and Agriculture Organisation (FAO) to initiate plans to set up, in West Africa, a network for forecasting and monitoring invasions, similar to that established in eastern Africa some 25 years ago (Odiyo, 1979; Lambert, 1989).

It is recognised though (Lambert, 1989) that reports of armyworm outbreaks have increased during the last decade due partly to the development of more efficient crop protection services, generally, in West African countries. Other possible reasons are the greater recognition of armyworm as a pest of cereal crops and an increase in the areas both of land used for agricultural purposes and of grassland savanna resulting from deforestation.

Table 1.1 Reported incidence of armyworm outbreaks in Ghana : 1937-1991

YEAR	REGION	AREA/CROPS AFFECTED		PERIOD[1]
1937	Eastern	*		
1950	Western	*		
1975	Ashanti	*		
1977	Eastern	*		
	Volta	*		
1978	Ashanti	*		
1980	Ashanti	*		
	Northern		maize[a]	R.S.
1981	Northern		maize	R.S.
1982	Ashanti	3 ha-	maize	M.C.S
	Northern		maize	R.S.
1983	Eastern	*		
	Brong-Ahafo		Maize, rice	
	Northern		Maize	R.S.
1984	Ashanti	6 ha-	Maize	
	Northern		maize	R.S.
1985	Northern		maize	R.S.
1986	Brong-Ahafo	*		
	Northern		maize	R.S.
1987	Ashanti	78 ha[c]		
	Brong-Ahafo	*		
	Eastern		maize	M.C.S.
	Northern		maize	R.S.
	Upper (East and West)	10000 ha-	maize, millet, rice, sorghum	R . S
	Western	190 ha-	rice	.

Table 1.1 continued

YEAR	REGION	AREA/CROPS AFFECTED	PERIOD
1988	Ashanti	168 ha	
		8353 ha- guinea corn, maize, rice and grass	
	Brong-Ahafo	*	
	Eastern	195 ha- maize	
	Northern	maize	R.S
	Upper East	millet	
	Volta	545 ha- maize	
	Western	0.2 ha- grass	
1989	Ashanti	224 ha	
	Brong-Ahafo	1.0 ha-maize	M.C.S
	Eastern	207 ha-maize	
	Northern	*	
	Upper East	*	
1991	Ashanti	*	
	Brong-Ahafo	64 ha	
	Greater-Accra	40 ha	
	Upper-East	350 ha early millet	
	Volta	90 ha maize	
1992**	Greater-Accra	maize	

[1]. Period of occurrence of outbreak, where provided in original record from Ministry of Agriculture.

* area or crop affected not specified in record.

[a], area not recorded; [c], crop(s) not recorded

M.C.S. = minor cropping season (September to October in the south of the country)

R.S. = rainy season (July to August in the north of the country)

** An outbreak site visited by this author (May, during major rainy season in the southern half of the country) but not in official records at the time of collection.

1.1.1 Economic importance

As an agricultural pest, *S. exempta* is ranked as the most important armyworm in Africa, and is probably the most economically important species in the genus. By the scale of its attacks on crops, it is considered one of the most severe pests worldwide (Brown and Dewhurst, 1975). Damage is principally caused to the foliage of the food plants. Leaves are at first skeletonised, and young plants may be completely destroyed. Crop may be entirely lost or the yield so reduced as to be economically unprofitable. Replanting of crops is sometimes necessary; the success of the second crop then depends greatly on how much of the rainy season remains. Larvae may attack grazing land rather than crops. For instance, 73,000 ha of pasture was destroyed south of Sultan Hamud (Kenya) in March, 1977 (Odiyo, 1979). Such attacks may lead to heavy losses of stock, and denuded pastures may be predisposed to soil erosion. In extreme cases, famine could result from crop losses caused by armyworm outbreaks (Brown, 1962; Odiyo, 1979). The total area reported to have been infested in Kenya, Tanzania and Uganda shows a wide annual variation, ranging from 10 km^2 in 1967-8 to 24,000 km^2 in 1970-71 (Odiyo, 1979). In 1984-85, there were several hundred outbreaks in East Africa, affecting Burundi, Kenya, Tanzania. as well as in Ethiopia. The outbreaks in Kenya were described as "extensive and severe", estimated at over 60,000 km^2 at some point during the course of the upsurge (Pedgley *et al.*, 1989).

Odiyo (1979) estimates that in an infestation with 1.8×10^9 larvae, covering 65 km^2 at a density of 28 larvae m^{-2}, the sixth instar larvae alone would be

be expected to feed at an average rate of some 50 tonnes dry weight of herbage per day. This calculation is based on an earlier estimate (Brown and Odiyo, 1968) that one larva can consume 200 mg dry mass of maize leaves during the sixth instar. Odiyo (1979) reckons that 50 tonnes per day is equivalent to the rate of feeding of about 8,000 head of cattle. Betts (1976) states that African armyworms have repeatedly been observed at densities of 100 m^{-2} and occasionally up to 3,000 m^{-2} covering areas ranging from 'a few to tens' of square metres. Janssen (1993) estimates that an infestation covering over 20,000 km^2 at a density of 100 larvae m^{-2} will lead to the consumption of more than 5 million tonnes of green leaves.

Lambert (1989) provides some estimates of financial losses incurred by countries in West Africa as a result of infestation by *S. exempta*. In 1979, 35% of all rice growers in Sierra Leone lost their entire crop, and the country had to import rice worth 58 million U.S. dollars. In 1980, Guinea lost 120,000 ha of crops amounting to some 15% of the total 'cereal area'. This was estimated at 180,000 metric tons, equivalent to approximately 75 million U.S. dollars.

1.2 Phase polyphenism

The larvae of *S. exempta* exhibit phase variation (Faure, 1943). Iwao (1962) defines phase variation as "an ecological phenomenon in which marked changes occur in the morphology and function of an individual as a result of interactions with other individuals and in which such changes have an adaptive significance in the species life history". This definition implies that phase variation is dependent on density of

population (interactions with other individuals), a factor which is known to influence growth and development of several species of insects in their natural environment (Gruys, 1970). Key (1950) describes phase variation as " a type of polymorphism superimposed on variation due to independent environmental and genetic factors affecting the same characters". The term "phase variation" is often replaced with such other terms as "phase polymorphism" (eg Tojo, 1991; Fescemyer, 1993) or "phase polyphenism" (Gatehouse, 1986; Casey, 1993). Other terms like "larval polychromatism" (Johnson *et al.*, 1985) and "colour polymorphism" (Anazonwu and Johnson, 1986) have also been used to describe this phenomenon.

Polyphenism is defined as a form of plasticity (Windig *et al.*, 1994), and Casey (1993) considers the phase phenomenon as a case of phenotypic plasticity. According to Rhoades (1985), the changes associated with phase in locusts and in some Lepidoptera, must be the result of phenotypic plasticity because they occur without the intervention of selection. Polyphenism is therefore considered an appropriate term and will be adopted in this thesis.

Studies on the density-dependent phenomenon of phase began with the work of Uvarov (1921), who demonstrated that different 'forms' of the migratory locust, *Locusta migratoria*, which had hitherto been regarded as different species were, in fact, different phases of the same species. He designated the conspicuously coloured individuals normally found in swarming populations as the phase *gregaria* and the procryptic insects in scattered populations as phase *solitaria*.

In the three major genera of locusts- *Locusta*,

Schistocerca and *Patanga* (*Nomadacris*)- hoppers from crowded females tend to be darker in colour and larger. Generally, *solitaria* have uniform (frequently green) colouration, while *gregaria* have a black pattern on a yellow or orange background (Uvarov, 1966). These colour differences have been shown to be due to the presence of different types of pigment in the integument of the hoppers. In addition to the differences in colour, there are differences in behaviour and physiology between the phases, in both hoppers and adults (review by Uvarov, 1966).

The discovery of phase variation in locusts started off investigations for similar variation among other insect groups. Faure (1943) reported the occurrence of differences in colour pattern, that depended on density of population, in the larvae of *Spodoptera* (*Laphygma*) *exigua* (Hübner), *S. exempta* and in *S. abyssinia* Guen., referred to as *S. capicola* by Brown (1962). Density-dependent differences in larval colour occur also in several other noctuids including *Autographa* (*Plusia*) *gamma* (L.) (Long, 1953), *Spodoptera* (*Prodenia*) *litura* (Fabricius) (Zaher and Moussa, 1961), *Mythimna* (*Leucania*) *separata* (Walker) (Iwao, 1962), *Spodoptera littoralis* (Boisduval) (Hodjat, 1970), *Alabama argillacea* (Hübner) (Johnson *et al.*, 1985) and *Anticarsia gemmatalis* (Hübner) (Anazonwu and Johnson, 1986; Fescemyer and Hammond, 1988; Fescemyer, 1993).

Faure (1943) named the different phases or colour forms he found, as *solitaria*, *transiens* and *gregaria*, adopting Uvarov's (1921) terminology for locusts. However, Whellan (1954) considered the terms *solitaria* and *gregaria* unsuitable for armyworms, because there was no clear evidence of aggregation, and substituted

"passive phase" (for *solitaria*) and "active phase" (for *gregaria*). He introduced these terms to stress on the differences in behaviour in the field of predominantly *solitaria*- or *gregaria*-phase larvae, and to avoid the implication that *solitaria*-phase larvae occur only in low densities. Indeed, "passive phase" populations may exist at fairly high densities (sometimes even higher than in "active" populations) provided that the larvae feed within a thick mat of grasses and are not crowded during development (Rose, 1979). In spite of these objections by Whellan (1945), it has become usual to use *solitaria* and *gregaria* (eg Gatehouse, 1987; Gunn and Gatehouse 1985, 1987; Khasimuddin, 1981) and these terms will be used in this thesis.

In *S. exempta*, the colour of *solitaria* larvae ranges from pale green to brown, and the *gregaria* are velvety black in appearance. Occasionally, dark green forms occur among *solitaria*, which resemble the *gregaria*. Faure (1943) regarded the range of colours between the pale green and velvety-black extremes as the intermediate *transiens* phase. In the laboratory, phase change can be induced by rearing larvae under solitary or crowded conditions.

Iwao (1962) observed that the darkening of caterpillars at higher densities, was caused by the deposition of melanin in the cuticle. The ground colour (ranging from greenish-yellow to reddish brown) was determined by the colour of hypodermal pigments and was not affected by changes in density. On this basis, Iwao (1962) put forward a hypothesis that the actual colour of a particular larva may be determined by a combination of two components, one dependent on population density and the other on density-

independent hereditary and environmental factors. Long (1953), after studying the histological basis of larval colour in three lepidopteran species, also concluded that the hypodermis was responsible for the actual colour differences between solitary and crowded larvae, whilst the darkening of crowded larvae was due to the pigmentation of the cuticle.

Matthee (1946) reported that *solitaria* characters, in *S. exempta*, could be intensified by breeding the "*solitaria* line" for three generations, but there was no evidence of intensification of *gregaria* characters in the converse direction. Faure (1943) had not found any evidence for the inheritance of phase characters in *S. exempta*. In locusts, the phase transition begins in the first generation, but continued exposure to the appropriate conditions for two or more generations is more effective (Uvarov, 1966). However, the first change in density causes a greater change in colour than does the continuation of the new treatment in the following generations (Uvarov, 1966).

The stimulus or stimuli that operate in density effects have been difficult to identify (Gruys, 1970). Key (1950) states that in locusts, the extent to which *gregaria* characters develop appears to depend on the degree of stimulation between individuals, which in turn depends on population density. There is some evidence that in the Acrididae, visual and olfactory or vibratory stimuli are involved, while in stored-product beetles, density effects may be induced by contamination of the medium with excreta. In the Lepidoptera, non-specific mechanical stimulation among the larvae is considered as the main factor involved in the induction of phase change (Iwao, 1962; review

by Gruys, 1970).

According to Nolte (1974), later experiments have ruled out visual and auditory stimulation in locusts but have shown that olfactory or tactile stimuli could still be involved in inducing phase change. It has been proposed also that in locusts, transition to the *gregaria* form and aggregation are stimulated by an airborne 'gregarisation pheromone' (Nolte, 1974). This pheromone is excreted, and accumulates around roosting swarms. The pheromone is taken into the haemolymph, where it activates various biochemical processes to initiate the changes in morphological, physiological and behavioural characteristics which together constitute the complex that differentiates the *gregaria* phase from the *solitaria* (Nolte, 1974).

In Lepidoptera, there is evidence also to indicate endocrine effects on phase polyphenism. In *Mythimna separata*, it has been suggested that the brain-corpora cardiaca-corpora allata complex (Br-Cc Ca) and the suboesophageal ganglion secrete a hormone(s) that induces the pigmentation of the integument. This hormone, the melanization and reddish coloration hormone (MRCH) may be involved in the larval colour changes corresponding to the phase variations in several armyworm species, including *M. separata* (Ogura, 1975). Matsumoto *et al.* (1981) found that MRCH can be extracted from the heads of *Bombyx mori* L. (silkworm) moths, indicating that it may be widespread among moths.

The phases in the larvae of *S. exempta*, in common with locusts and other lepidopteran species like *M. separata* which exhibit phase variation, show differences in their physiology and behaviour. Simmonds and Blaney (1986) reported a shorter larval

period in the *gregaria* phase of *S. exempta*. In their experiments, the magnitude of the differences in development rates between the phases was affected by diet, being greater in larvae fed on maize or artificial diet than in those fed on wheat. Similar differences in rates of development have been found in other lepidopteran species. In *M. separata* (Iwao, 1962) and in *Autographa gamma* (Long, 1953), crowding reduces the mean duration of larval period and the individual variation in rate of development. However, Iwao (1962) observed that larval crowding retarded development in *Mythimna* (*Leucania*) *loreyi* (Duponchel).

In several species, activity or immediate behavioural response to certain stimuli is more intense when the larvae are crowded, while the solitary larvae show skulking behaviour and often feed at night (Gruys, 1970). Simmonds and Blaney (1986) observed that *solitaria* larvae of *S. exempta* spend more time feeding and resting than do crowded larvae, but they found no overall differences between the phases in time spent moving. Conversely, Long (1953) found that *gregaria* larvae of *A. gamma* spent more time in feeding and other forms of activity (including locomotion and irritation reactions) than *solitaria*. Similar observations were made on *M. separata* by Iwao (1962). It appears therefore, that at least some of the effects of phase on the biology of an insect are species-specific.

Brown (1962) notes that in armyworms, the effects of crowding mainly concern physiological and life history characteristics, with little evidence of the morphometric change which is a conspicuous feature of phase variation in locusts. Only an indefinite, qualitative difference in larval size has been

reported by Whellan (1954) who stated that mature *S. exempta* larvae tend to be more "slender" when reared under crowded conditions. There is no evidence of any phase differences in the moths (Rose, 1979).

1.2.1 The role of phase in *S. exempta* life history

According to Iwao's (1962) definition for phase variation, the changes associated with this phenomenon should "...have an adaptive significance in the species life history". It is therefore necessary to establish the functional significance of phase polyphenism in the life history of *S. exempta*.

In the early years of research on this species, there was much controversy on the role of migration in its biology. Hattingh (1941) proposed that populations built up rapidly from low densities to cause outbreaks, and that this build up was encouraged by a delayed start to the rainy season in southern Africa. He considered it unnecessary for moths to migrate from one area to cause an outbreak in another. On the other hand, Faure (1943) was convinced that larvae in outbreaks were produced by moths which migrated from permanent breeding areas, where the species could breed throughout the year. He argued that such permanent breeding grounds were necessary for the survival of the species, as it did not seem to have a quiescent stage to go through adverse environmental conditions like the winter of southern Africa.

With the recognition that the relative importance of migration or of local build-up of populations was critical to the development of a control strategy, this issue has been the subject of further research. Brown *et al.* (1969) analysed the data on outbreaks and

moth catches in light traps in East Africa, and obtained a pattern which suggested that there was a seasonal movement of outbreaks northwards from Tanzania to Ethiopia and the Yemen. The movement was associated with the rainy season in each country, which also depended on the movement of the Inter-Tropical Convergence Zone (ITCZ). The geographical progression of outbreaks occurred at approximately one generation intervals.

Riley *et al.* (1983) observed the flight of moths emerging from an outbreak site, using radar and infra-red optical equipment. They demonstrated that downwind (migratory) flights could cover at least 20 km. Riley *et al.* (1983) observed, however, that moths dispersed rapidly during flight, unlike locusts which remain cohesive in swarms. From this, they deduced that the large numbers of moths which lead to outbreaks must be a consequence of reconcentration, a suggestion favoured by Rose *et al.* (1985).

In a mark-and-recapture experiment (Rose *et al.*, 1985), marked moths were captured up to 147 km from the emergence site and up to 90 km after flying for only one night. Rose *et al.* (1985) concluded that the moths depend on winds to achieve considerable displacements. These studies provide ample evidence for migration by armyworm moths and for the involvement of migration in initiating outbreaks of *S. exempta*.

If outbreaks are caused by immigrant moths, then it is necessary to identify the sources of the moths which cause the first outbreaks of a season, that is, where the moth populations occur in between outbreaks. There is as yet no evidence for permanent breeding grounds in the sense suggested by Faure (1943). The

current view is that during the 'off-season' (the period between outbreaks), low-density populations persist in certain areas which have green vegetation as a result of erratic out-of-season rainfall (Rose, 1979; Gatehouse, 1987). Moths from these populations, believed to be in the *solitaria* phase, give rise to the first outbreaks of a season, termed 'primary outbreaks' (Rose *et al.*, 1987). Migrant moths from primary outbreaks are concentrated in certain localities by seasonal convergent winds associated with rainfall, to cause 'secondary' outbreaks. Outbreaks which have the potential of leading to other outbreaks, are termed 'critical' (Rose *et al.*, 1987; Gatehouse, 1987; Pedgley *et al.*, 1979; Tucker, 1994).

In locusts, the most important feature of the phase change (in economic terms) is the tendency for aggregation in the phase *gregaria* resulting in swarming behaviour, greater mobility, and higher, more synchronised activity followed by large-scale migrations and invasions (Brown, 1962). There is thus a relation between phase change and a tendency to migrate. Iwao (1962) considers such features as reduced body weight and wing loading, lower water content and increased life-span of moths produced from *gregaria* larvae of *M. separata* as adaptations for long range migration. Brown (1962) observed that some of the characteristics of the *gregaria* phase in armyworms may favour higher rates of population growth, and hence outbreaks, but that the lack of clear evidence for aggregation (unlike in locusts) did cast some doubt on the relationship between phase variation and migration in armyworms.

In addition to the possible effects of phase on rate of larval development and activity in *S. exempta*,

previously cited, there have been other attempts to identify morphological or physiological changes associated with phase, and to assess their possible influence on migratory capacity. Parker and Gatehouse (1985a) reported that flights in moths from larvae reared at high densities were significantly longer than those of moths from larvae reared at lower densities. Selection experiments (Parker and Gatehouse, 1985b) also indicated a genetic component in the determination of flight capacity, which made it difficult to attribute the earlier differences in flight potential (Parker and Gatehouse, 1985a) to the effect of rearing density alone. These doubts appear to have been cleared by Woodrow *et al.* (1987) who demonstrated a higher flight potential in female moths obtained from *gregaria* larvae of *S. exempta* compared with siblings reared in isolation. There is also evidence that the lipid content of the body is higher in individuals in the *gregaria* phase, which also suggests that individuals in this phase are better adapted for flights over longer distances (Matthee, 1945; Gunn and Gatehouse, 1987).

Gruys (1970) is of the view that the ability to respond in some way or other to changes in population density can be of survival value and can thus give selective advantage. A useful response is to escape density-related mortality either in the same generation or to avoid high mortality among the progeny, which is one of the benefits of migration. According to Gatehouse (1987), capacity for migratory flight in *S. exempta* is inherited, and that larval phase modulates this trait in response to changes in the quality of the habitat. The same author considers *S. exempta* to be adapted for survival at low densities

(that is in the *solitaria* phase). He concludes that the *gregaria* phase evolved under intense selection pressure from limiting food resources and natural enemies (including disease) at high densities to increase development rate and subsequent dispersal through migration which will lead to low densities in the ensuing generation.

1.3 Objectives of study

This project will investigate some of the possible effects of phase polyphenism on the physiological ecology of *S. exempta*. The study covers three main areas as outlined below.

1. Effects of larval phase on aspects of reproduction by female moths.

2. Relationship between phase and food consumption and utilisation by larvae: since allocation of resources in the adult may depend on how much energy is accumulated during the larval stage, it is necessary to study the effect of population density, and hence phase, on feeding rates and the efficiency of utilization of food.

3. Effect of solar radiation on the behaviour and development rates in the two phases: to test the hypothesis that the dark pigment in the *gregaria* increases absorption of radiant energy, boosting body temperatures and accelerating development (Rose, 1979; Gatehouse, 1986).

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CHAPTER TWO

GENERAL MATERIALS AND METHODS

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2.1 Laboratory conditions

Experiments were performed with moths and larvae from a culture kept in rooms with regulated conditions. The temperature in the rooms was maintained at 26 ± 2°C, and the relative humidity at 60-70%. The photoperiod regime was 12L:12D.

2.2 Insect material

The insects in the culture were obtained from the field during outbreaks in Kenya or Tanzania. Some material was also obtained from laboratory cultures kept at the Wageningen Agricultural University in the Netherlands. Moths obtained at any time from any of these sources were kept isolated from the existing culture for at least one generation, to ensure that they were free from disease, before they were cross-mated with the moths in the main culture. Thus new genetic material was introduced into the culture as and when it became available from the field or from other laboratories.

2.3 Containers for larvae and adults

Two types of glass jars were used for rearing larvae; 500ml Kilner jars and 120ml glass jars. Both types of jar were used for rearing larvae, but moths were kept only in the 500ml jars.

When in use, each jar of either size had filter paper lining the base and as a cover. The latter were held in place by plastic screw-on tops.

2.4 Egg collection

Moths were kept in pairs, male and female, in 500 ml Kilner jars with crumpled filter paper to provide shelter for moths, particularly for the ovipositing female. Where eggs were laid on this "refuge" or on the filter paper at the bottom or top of the jar, they were collected daily and transferred into small plastic pots (55 ml) to await hatching. Occasionally eggs were laid instead on the wall of the glass jar or on the containers holding the water or sucrose solution, in which case they were left to hatch in the jars and the moths transferred to fresh jars.

2.5 Rearing Density

When the eggs hatched in the 55 ml plastic pots, they were fed a little piece of fresh maize leaf and then transferred to Kilner jars within 24 hours. The number of larvae was subsequently reduced to eight per jar by the third instar, when they were to be kept as *gregaria*. Usually, *gregaria* larvae were kept at this density, but for some experiments, smaller numbers were reared in smaller containers (see subsequent chapters for details).

Solitaria larvae were reared in 120 ml glass jars into which they were separated within 24 hours of hatching.

2.6 Feeding

Larvae were fed daily with fresh maize leaves taken from potted plants grown in a greenhouse. The cut ends of the strips of leaf were immersed in distilled water

in glass vials, in the Kilner jars, or small plastic vials in the 120 ml jars. These vials were then plugged with cotton wool to prevent the larvae from drowning.

Each day, food left over from the previous day was removed together with the frass, before fresh food was provided. The filter paper lining the base and top of the jars was also replaced at a frequency depending on the age and health of the culture. Replacement was more frequent for older larvae because they produced more frass. Where the culture showed signs of viral infection (see below) it was necessary to change filter paper linings (or even provide fresh jars) daily.

Moths were fed distilled water or 10% sugar solution (w/v) held on cotton wool in plastic vial stoppers.

2.7 Sanitary Precautions

A major problem with the maintenance of *Spodoptera exempta* in the laboratory is the susceptibility of the larvae to the nuclear polyhedrosis virus. A number of measures were taken to reduce the incidence of infection and to check its spread whenever it occurred.

Any larvae suspected of having an infection were immediately taken out of the culture. Such larvae were sluggish or unable to feed. In the latter stages of infection, clear or milky fluid exuded from the bodies of the larvae. Where the level of infection was very high, entire jars of larvae were removed and sterilised by keeping them in a hotbox oven at 150°C for at least 24 hours. All used glass jars and vials

were similarly sterilised in the oven. When these were removed from the oven, they were washed in soap and hot water and again kept in the oven to dry and be further sterilised. Plastics were kept in 0.5% solution of bleach (sodium hypochlorite) for at least 24 hours to sterilise them before washing and drying. Prior to setting up a new batch of larvae in the culture room, and at regular intervals during their growth, the benches (on which the jars were placed) were also wiped with bleach solution.

At one stage during this project, when the survival of the entire culture was threatened with disease, the rearing of larvae in groups was suspended. For one generation, only solitary larvae were kept so that diseased larvae could be eliminated with a lower risk of infecting others.

2.8 Terminology

The terms 'first' and 'third generation' *solitaria* are used in subsequent chapters to describe changes in rearing density between generations of insects in the culture.

i) first generation refers to *solitaria* larvae which were obtained from moths which were themselves reared in the larval stage as *gregaria*.

ii) third generation refers to *solitaria* larvae obtained from moths whose grandparents had first been reared as *solitaria* in the larval stage.

The two categories were used in some experiments to assess the relation between the expression of *solitaria* characteristics and the number of generations that the insects had been reared as *solitaria*.

Where the term *solitaria* or *gregaria* is applied to moths, they only indicate the rearing density of the larvae from which the moths were obtained. These terms do not refer to morphological or behavioural differences in the adults.

CHAPTER THREE

EFFECT OF LARVAL REARING DENSITY AND ADULT DIET ON FECUNDITY AND OTHER LIFE-HISTORY TRAITS IN *SPODOPTERA EXEMPTA*

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3.1 INTRODUCTION

3.1.1 Effect of Nutrition on Fecundity

It is known for a variety of insects that feeding is essential for egg maturation, increased egg production and oviposition. The effects of food result from a balance between several factors such as food quantity, quality and specific requirements, temperature, and metabolism (Engelmann 1970). The same author considers that nutrition is probably the most important single factor that affects reproduction in the majority of insect species and that the effects of other external factors (light, temperature and humidity) are indirect, through their effect on feeding and mating activities. The effects of different kinds of food may be due to differences in relative amounts ingested or of physical or chemical differences in the food consumed (Johansson, 1964).

The significance to reproduction of nutrients obtained by adult female Lepidoptera range from complete autogeny, where adults do not feed, to complete anautogeny, where feeding is required prior to oviposition (Carrol and Quiring, 1992). In some species, all the materials required for reproduction are accumulated in the larval or nymphal stages. Fecundity depends mainly on larval nutrition in these and in species in which the adults do not feed (Johansson, 1964; Engelmann, 1970). In other species, the utilisation of reserves carried over from pre-imaginal stages for reproduction and other activities, depends on energy derived from the adult diet. Adult feeding is necessary in such species (Johansson, 1964). Lukefahr and Martin (1964) reported that adult

feeding was not essential for mating or oviposition in the bollworm, *Helicoverpa* (*Heliothis*) *zea* (Boddie), if larval feeding was adequate. The tobacco budworm, *Heliothis virescens* (Fabricius), and the cotton leafworm, *Alabama argillacea* were found to be more dependent on adult food. However in the bollworm, adult feeding did increase fecundity regardless of larval diet. In *Epiphyas postvittana* (Walker), fecundity depends mainly on the quality of larval food, if water is provided from emergence (Gu and Danthanarayana, 1990). According to Gunn and Gatehouse (1985), *Spodoptera exempta* did not require adult feeding for maximum egg production provided the larvae had been fed *ad libitum*.

In addition to the provision of energy and materials for reproduction, pre-imaginal diet may affect the number of ovarioles which in turn influences the number of eggs produced. Dietary deficiencies could also disturb yolk synthesis, an important stage in egg production (Johansson, 1964).

3.1.1.1 Importance of water

A number of laboratory studies have demonstrated that water is essential for egg production in some lepidopteran species. Egg production increased three to four times in *Ostrinia* (*Pyrausta*) *nubilalis* (Hübner) (Kozhantshikov, 1938) and tripled in *Agrotis orthogonia* Morrison (Jacobson, 1965) when drinking water was allowed. For *O. nubilalis*, Kira *et al.* (1969) also reported that drinking water was essential for 'good production of eggs', and also increased hatchability of egg masses. In *Ephestia cautella* (Walker) and *E. elutella* (Hübner), egg

production increased after water was supplied. The effect of drinking water on fecundity was less marked in *E. kuhniella* Zeller than in the other two species. This might be because the latter species is almost entirely restricted to flour mills where there is no supply of adult food or water (Norris, 1934). When adult *Spodoptera exempta* were not provided with water, they either failed to lay eggs at all, or laid less than moths that were given water (Gunn and Gatehouse, 1985). In the pyralid, *Parapediasia teterella*, adults do not feed, but water is important both to survival and lifetime reproduction (Marshall, 1990).

Water is probably essential for egg production because eggs contain a considerable amount (30-80%), while female adults do not have large water reserves (Engelmann, 1970). For *S. exempta*, Gunn and Gatehouse (1985) concluded that access to water was necessary for hydration and maturation of oocytes and achievement of potential fecundity. Results of experiments on *Periplaneta americana* (L.) (Verret and Mills, 1975) and *Manduca sexta* (Johannsen) (Nijhout and Riddiford, 1979) also suggest that water is necessary for hydration of oocytes prior to chorion formation. Similarly, Gu and Danthanarayana (1990) reported that *Epiphyas postvittana* can lay fertile eggs without feeding but they required water to achieve their full reproductive potential.

3.1.1.2 Carbohydrates

An adequate supply of carbohydrates is also a specific requirement for reproduction in many insects. Carbohydrates do not seem to be directly utilised in yolk formation in oocytes, as eggs contain very little

of them. Rather, their primary role is possibly the provision of energy for the utilization of dietary protein reserves in both autogenous and anautogenous species (Johansson, 1964; Engelmann, 1970).

Carbohydrates can affect fecundity indirectly by prolonging lifespan and they may affect the quantity of food consumed (Johansson, 1964), probably by acting as phagostimulants. However, as Norris (1934) found in two species of *Ephestia*, and Zaher and Long (1959) in *Autographa gamma* (L.), longer life does not necessarily mean producing more eggs. Stern and Smith (1960) observed that female *Colias philodice eurytheme* Boisduval that were fed on sugars laid three times more eggs than those which had only water. The concentration of sugars in the food may also influence fecundity. Shorey (1963) reported a positive correlation between total egg output and dietary sucrose concentration in *Trichoplusia ni*: on 1-4% sucrose, 200-400 eggs were laid by a female; on 8-14%, approximately 500-600 eggs were laid.

More recent studies on other lepidopteran species have confirmed this general observation that fecundity is increased when sugar is provided. Sugars in the adult diet increased fecundity, longevity and the ability to maintain adult weight in the checkerspot butterfly, *Euphydryas editha* (Murphy *et al.*, 1983) . Similar results have been obtained for the pine beauty moth, *Panolis flammea* (Denis and Schiffermüller) (Leather, 1984) and the common imperial blue butterfly, *Jalmenus evagoras* (Hill and Pierce, 1989). Mason *et al.* (1989) also studied effects of adult diet and flight on a number of traits, including fecundity, in the soybean looper, *Chrysodeixis* (*Pseudoplusia*) *includens* (Walker). They

found that non-flown, starved moths (in this case, moths fed on water only) laid a significantly smaller number of eggs than non-flown moths which were fed on 15% sugar solution. For the same species, Jensen *et al.* (1974) reported that fecundity increased when moths were provided with sucrose or honey solution. Sucrose solution increased fecundity in *Zeiraphera canadensis* Mutuura and Freeman (Carrol and Quiring, 1992), and in *Heliothis virescens* and *Alabama argillacea* (Lukefahr and Martin, 1964). In the latter species, it was found that adults needed food for a minimum of two days for optimum mating success, egg deposition and egg viability. Diets with sugars significantly increased fecundity in *Ostrinia nubilalis* (Leahy and Andow, 1994).

This relation between sucrose-feeding and fecundity in moths is not without exception or interaction with other factors. Gunn and Gatehouse (1985) did not find a significant difference between fecundity in water-fed and sucrose-fed *S. exempta* moths, though their results indicated that 'sucrose in the adult diet can increase fecundity, particularly in lighter moths from a suboptimal larval feeding regime'. However, the same authors (1988) in another experiment on the effect of flight and adult diet on fecundity in *S. exempta*, reported that the provision of sucrose increased fecundity for unflown (control) moths. In *Epiphyas postvittana*, Gu and Danthanarayana (1990) found no significant difference in fecundity between moths fed on a 30% solution of honey and those that were given only water immediately after emergence. However, if moths were starved for three days before water or honey was provided, then moths provided honey were more fecund.

3.1.2 Effect of population density (phase) on fecundity

The effect of population density on the rate of oviposition is more variable between species than the effect of diet. In adult insects, there is evidence that animals in isolation, or in pairs, had a higher total egg output than did crowded ones (Engelmann, 1970). In *Drosophila simulans* Sturtevant (Sameoto and Miller, 1966), females living in groups lay fewer eggs than do solitary individuals, even with adequate food. Though *Schistocerca gregaria* (Forskål) females matured eggs at a faster rate when crowded, their total egg output was below that of isolated females. In *Patanga* (*Nomadacris*) *septemfasciata* (Serville) isolated pairs produced nearly twice as many eggs in a life-span as did crowded females. The effect of crowding of females has a similar effect on fecundity in *Locusta migratoria migratoroides* (Reiche and Fairmaire) (Norris 1952, 1959; Uvarov, 1960).

The density of adult insects does not just affect the total lifetime fecundity, but also the fecundity per unit of time (Peters and Barbosa, 1977). According to Engelmann (1970), the probable reason why fecundity is lower in grouped animals in these species is that individuals interfere with one another in feeding, which is an essential factor in reproduction. Crowding may interfere with other activities of the adult female, such as resting, pairing and grooming, which also reduce egg production. The anatomy of the reproductive system itself could be affected too. For instance, when female *Patanga* were reared in relative isolation for several generations, more ovarioles developed per ovary than when the females were reared

in large groups (Norris, 1959).

In locusts, fecundity appears to be affected to a great extent by crowding in the adults, as the references cited indicate. Phase differences are less obvious in adult Lepidoptera than in locusts, so this study will focus on how crowding in the larval phase affects reproduction in the adult.

In *Helicoverpa armigera*, Tripathi and Singh (1989) found that larval crowding reduced fecundity of the adults. High larval rearing density also reduced the weight of female pupae and, subsequently, fecundity in the elm spanworm, *Ennomos subsignarius* (Hübner) (Drooz, 1966) and in *Bupalus pinarius* (L.) (Klomp, 1966; Gruys, 1970). In *Pieris brassicae* (L.), solitary females had a greater egg-laying capacity than those from crowded conditions (Zaher and Long, 1959). Danthanarayana *et al*. (1982) reported an inverse relation between larval density and fecundity of the resulting moths, in *Epiphyas postvittana*. No difference in fecundity was detected between moths from low and high larval densities in *Mythimna separata*, *M. loreyi* or *Leucania placida*, but in *Naranga aenescens* Moore and in *Parnara guttata* (Bremer and Grey), fecundity was reduced at high densities (Iwao, 1962). For *Spodoptera* (*Prodenia*) *litura*, Zaher and Moussa (1961) did not find any phase effect on fecundity (higher in the *gregaria* but not significantly so), but Rivnay and Meissner (1966) observed a reduction in fecundity at high densities in this species due to a higher rate of egg production throughout the life of the *solitaria*. A review of phase effects on fecundity indicates that in many of the species studied, high larval density reduces fecundity, but it has no effect on fecundity in

Agrotis ipsilon (Hufnagel), while it causes an increase in fecundity in *Bombyx mori* (L.) (Gruys, 1970). For *Mamestra* (*Barathra*) *brassicae* (L.), Hirata (1956) recorded an increase in fecundity with increase in larval density, while Bonnemaison (1962; cited by Gruys, 1970) reported a reduction. In *Autographa gamma*, females from crowded cultures laid more eggs than those from solitary cultures; on the average, 15% more (Zaher and Long, 1959). A difference in the same direction was reported for *Spodoptera exempta* by Gunn and Gatehouse (1987), but the difference was even larger and depended on diet. Females from *gregaria* larvae laid approximately twice the number of eggs produced by *solitaria* moths when both were given distilled water only. When fed sucrose the difference between phases was eliminated.

3.1.3 Effect of phase and diet on other life history traits

Longevity

Many of the laboratory studies on the effects of diet on fecundity also include its effects on longevity. Female *Euphydryas editha* which were fed diets containing sugar, had significantly greater longevities than those fed just water or water and amino acids (Murphy *et al.*, 1983). Sucrose-feeding also increased adult longevity in *Ostrinia nubilalis* and *Agrotis segetum* (D&S) (Kozhantshikov, 1938), *Chrysodeixis includens* (Jensen *et al.*, 1974) and *Zeiraphera canadensis* (Carroll and Quiring, 1992). In *Jalmenus evagoras* (Hill and Pierce, 1989) individuals fed 25% sugar had the greatest longevities. Sugar in

the diet nearly doubled longevity in *Helicoverpa zea, H. virescens* and *Alabama argillacea* (Lukefahr and Martin, 1964). Gu and Danthanarayana (1990) also reported that longevity in *Epiphyas postvittana* depended on the availability of honey or water, honey-fed moths surviving for an average of 5 days longer than water-fed. The latter also lived 4 days longer than moths that were denied both water and honey. However, denying moths water or honey for the first three days after emergence did not significantly reduce longevity. This led the authors to conclude that early mortality of starved moths was due to dehydration rather than exhaustion of metabolic reserves. A similar conclusion was drawn by Gunn and Gatehouse (1985) who also reported that *S. exempta* females fed sucrose lived significantly longer than those in the distilled-water group.

In both *Autographa gamma* and *Pieris brassicae,* females from crowded cultures lived longer than those kept under solitary conditions (Zaher and Long,1959). In *Mythimna separata,* Iwao (1962) states that moths from 'high density' cultures lived about a day longer than 'low-density type' moths, but he gives no indication of the level of significance of this difference. Feeding on honey increased the difference between phases to over three days, if the moths were kept singly. When moths were paired, the difference between phases was reduced, mainly as a result of shortening of the lifespan in high-density type individuals. The same author did not find any effect of high density on longevity in *M. loreyi* or in *Naranga aenescens,* but in *Parnara guttata* crowding reduced longevity. Rivnay and Meissner (1966) also found a greater longevity in the *solitaria* in

Spodoptera littoralis as did Zaher and Moussa (1961). Phase did not influence life-span in *Bupalus pinarius* (Gruys, 1970) or *Mamestra brassicae* (Hirata, 1956).

In the three major species of locust, the effect of phase on longevity is related to its effect on the rate of sexual maturation. The higher the density, the more rapidly *Schistocerca gregaria* adults mature; maturation time in crowded *Patanga* adults, also tends to be shorter than adults kept in single pairs. In both species, crowding of adults increases longevity. On the other hand, *solitaria* in *Locusta migratoria* mature more rapidly than the *gregaria*, which live longer (Norris, 1952, 1959). Thus, the phase that matures more slowly tends to have a longer life-span. Uvarov (1966) proposes that this tendency may be connected with differences in neurosecretory activity between the species.

Oviposition period

In *Anthrenus verbasci* (L.) (Blake, 1961), oviposition period was increased by feeding on sugar. Enhanced fecundity of sucrose-fed female *Z. canadensis* was due to increased longevity and longer oviposition period (Carroll and Quiring, 1992). Diets with sugars also increased adult longevity and oviposition period in *O. nubilalis* (Leahy and Andow, 1994).

Iwao (1962) reported that there was no difference in mean oviposition period between the phases in *Mythimna separata*, but the oviposition period was shorter in the *gregaria* in *N. aenescens*. Lifespan and oviposition period were reduced in the *solitaria* phase in *Spodoptera exempta*, when moths were provided with only water (Gunn and Gatehouse, 1987).

Pre-oviposition period (POP)

A tendency for the *gregaria* to have a shorter POP has been observed in *Autographa gamma*, while in *Pieris brassicae*, it was shorter in the *solitaria* (Zaher and Long, 1959). The reliability of this report on *P. brassicae* is however doubtful, because it is not based on data for individuals, but on 'consideration of available figures' from 30 pairs of butterflies per cage. Larval crowding prolonged the pre-reproductive period in *Mythimna convecta* (McDonald and Cole, 1991). In *M. separata*, Iwao (1962) found the average length of the pre-oviposition period to be only marginally longer in high-density type moths (the difference was not significant). In fact, in the same paper, Iwao refers to another experiment on the same species, in which equal POP was recorded for the two phases. The POP was shorter in solitary cultures in *N. aenescens* (Iwao, 1962), but longer in *S. litura* (Zaher and Moussa, 1961). The latter agree with Rivnay and Meissner (1966) who found a 'slightly' longer POP in moths reared from solitary larval cultures. POP was unaffected by phase in *S. exempta* (Gunn and Gatehouse, 1987).

3.1.4 Objectives of study

(1) To investigate the effects of larval density (phase) and adult diet on:

i) fecundity,

ii) pre-oviposition period,

iii) oviposition period,

iv) number of egg batches

and v) longevity of female *S. exempta* moths, with modifications to the larval rearing conditions and method of data analysis used in previous studies (Gunn and Gatehouse, 1987).

(2) To study how the number of generations for which a *solitaria* line is kept (Matthée, 1946; Brown, 1962) affects the expression of the traits listed in 1 above.

3.2 MATERIALS AND METHODS

This experiment was carried out in two parts; one using first-generation *solitaria* and the other using third-generation *solitaria*, as described in Chapter Two.

3.2.1 First Generation

Larvae were reared in 250ml plastic dishes with filter paper at the bottom and with plastic tops, each top perforated with pin holes. The same size dish was used for both phases, reared at 1 per dish for *solitaria* and 4 per dish for *gregaria*. Larvae were separated into these two categories within 24-36 hours after hatching. All larvae were fed an excess of maize leaf daily. The maize leaves had their cut ends kept in plastic vials of water to keep them turgid, and the vials were plugged with cotton wool to prevent the larvae from drowning. The dishes were cleaned daily before feeding and the filter paper floors or the dishes themselves were replaced when necessary. The environmental conditions were thus kept uniform for both *solitaria* and *gregaria* larvae. The temperature in the room during this experiment was 26 ± 2°C, the relative humidity was 55-65% and the photoperiod regime 12L:12D.

The pupae were sexed 24-36 hours after pupation when the cases were hard enough to prevent damage to the developing moths. They were then placed individually in 55ml plastic pots with perforated lids, till emergence of adults. Moths were weighed as pharate adults 1-6 hours prior to eclosion when the pupal cases looked very dark. Weighing during this

period avoids the fluctuations in weight, due to voiding of meconium, associated with weighing newly emerged moths. The weight of the pupal case just before emergence has been shown to be less than 1.5% of total weight (Gunn and Gatehouse, 1985).

When moths emerged, they were crossed with males that were a day or more older if possible, or at least of the same age. Within 12 hours after emergence the moths were paired up in 500ml Kilner jars with filter paper tops, floor and refuge as described in Chapter Two. Both *solitaria* and *gregaria* moths were divided into 2 groups, one fed on distilled water and the other on 10% sucrose (w/v). Thus there were four groups or treatments:

i. *solitaria* fed distilled water.
ii. *solitaria* fed sucrose.
iii. *gregaria* fed distilled water.
iv. *gregaria* fed sucrose.

The water or sucrose supply, which was renewed daily, was held in plastic containers on wicks of cotton wool. The jars were checked for eggs during cleaning of jars and renewal of food or water supply. Fecundity was recorded as the total number of eggs laid by each female. In all, data were collected for 331 females, made up of 192 *solitaria* and 139 *gregaria*. Ninety-six (96) *solitaria* and 69 *gregaria* were fed distilled water while 96 *solitaria* and 70 *gregaria* were fed on sucrose. The differences in sample size for the different treatments was due to differential mortality, mainly from viral infection (in all, 330 *solitaria* and 336 *gregaria* were reared). The moths used in this experiment were obtained from eggs from 9 females, and the data were collected separately for the resulting families. In each family, the larvae for

the experiment were reared from the same batch of eggs. Therefore, the *solitaria* and *gregaria* larvae were siblings, thus reducing differences due to genetic factors. Again due to differences in mortality, the nine families are not equally represented in the data set.

3.2.2 Third Generation

The procedure was the same as that described for the first generation moths with the following exceptions:

1. Eggs were obtained from four families.
2. Larvae were maintained in 120 ml glass jars with filter paper roof and floor to reduce condensation. In spite of the pin-hole perforations, there was some condensation in the plastic containers in Part 1 (section 3.2.1). Though there was no direct evidence, it was suspected that the excess moisture could have had adverse effects on the larvae, hence the replacement of the containers with glass ones.
3. *Solitaria* were obtained from a line kept solitary for at least three generations.

In addition to fecundity, data were also collected for each female (in both first- and third-generation) for the following traits associated with reproduction:

i. Longevity
ii. Pre-oviposition period
iii. Oviposition Period
iv. Number of egg batches.

3.2.3 Statistical Analyses

An analysis of covariance (ANCOVA) was performed on the fecundity data using the MANOVA procedure of the SPSS statistical package with body weight as the covariate. A similar procedure was used in the comparison of the other traits where there was an indication of a significant correlation with weight (see Appendix to Chapter Three).

3.3 RESULTS

In all tables in this section, these symbols are used to indicate the level of significance of the F ratios from the ANOVA or ANCOVA:

* = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$,

*** = $P \leq 0.001$, ns = not significant.

3.3.1 Fecundity

The results of the analysis of variance of fecundity (adjusted for weight) for the first-generation experiment are summarised in Table 3.1.
The *gregaria* moths laid about 26% more eggs than *solitaria* (P= 0.019), when the moths were fed distilled water only. When sucrose was provided, the difference in fecundity between phases was not significant.

The effect of diet is more consistent: in both *solitaria* and *gregaria*, sucrose-fed moths laid significantly more eggs than moths fed distilled water only.

Table 3.2 gives the fecundities for the experiment in which third-generation *solitaria* females were used. There was no significant effect of larval phase on fecundity (combined means ± se: all *solitaria*, 789.0 ± 49.6; all *gregaria*, 688.7 ± 33.4).

The effect of diet is similar between the two experiments in that sucrose-feeding increased fecundity but, in the third-generation experiment, fecundity was independent of diet in the *gregaria*. In the first-generation experiment, the increase in fecundity due to sucrose-feeding was significant in both phases.

Table 3.1 Effect of larval phase and adult diet on weight-related fecundity of female *Spodoptera exempta* fed either 10% sucrose or distilled water, using first-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	599.5 ± 29.3 a (96)	757.2 ± 29.9 b (69)
Sucrose	845.9 ± 30.2 c (96)	949.3 ± 36.6 c (70)
	Phase effect: $F_{1,294}$ = 5.56 *	
	Diet effect: $F_{1,294}$ = 35.68 ***	

Table 3.2 Effect of larval phase and adult diet on weight-related fecundity of female *Spodoptera exempta* fed either 10% sucrose or distilled water, using third-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	640.7 ± 58.0 a (28)	657.6 ± 40.9 a (35)
Sucrose	937.3 ± 64.7 b (27)	702.0 ± 49.2 a (36)
	Phase effect: $F_{1,109}$ = 2.15 ns	
	Diet effect: $F_{1,109}$ = 10.00 **	

Table 3.3 Effect of larval phase and adult diet on weight-related number of egg batches produced by female *Spodoptera exempta* fed either 10% sucrose or distilled water, using first-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	3.2 ± 0.09 a (96)	3.3 ± 0.12 a (69)
Sucrose	4.5 ± 0.12 b (96)	4.4 ± 0.17 b (70)
Phase effect:	$F_{1,294}$ = 0.00 ^{ns}	
Diet effect:	$F_{1,294}$ = 113.67 ***	

Table 3.4 Effect of larval phase and adult diet on number of egg batches produced by female *Spodoptera exempta* fed either 10% sucrose or distilled water, using third-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	4.2 ± 0.33 a (28)	4.0 ± 0.21 a (35)
Sucrose	5.2 ± 0.40 a (27)	4.5 ± 0.39 a (36)
Phase effect:	$F_{1,110}$ = 2.44 ^{ns}	
Diet effect:	$F_{1,110}$ = 5.54 *	

3.3.2 Number of egg batches

There was no effect of larval phase on the number of egg batches in the first-generation experiment (Table 3.3). This means that though the *gregaria* laid more eggs than *solitaria*, they did not produce more batches, implying that the *gregaria* laid larger egg batches than the *solitaria*.

Sucrose-fed moths produced more egg batches than water-fed moths in both phases (Table 3.3).

There was no effect of larval phase on the number of egg batches in the third-generation experiment either. For the effect of adult diet, the ANOVA indicates that sucrose-feeding increased the number of egg batches significantly. This looks particularly so from the means for the two diets within the *solitaria* (Table 3.4). However, the pairwise comparison by the Scheffe test does not reveal any significant differences due to diet within either phase.

3.3.3 Oviposition Period

In the first-generation experiment, larval phase had no significant effect on oviposition period (Table 3.5), which is why the difference in number of egg batches between phases was not significant. This is a further indication that in this experiment, the phase difference in fecundity, on distilled water is a result of the *gregaria* producing larger egg batches in relation to body weight.

Sucrose in the adult diet increased the oviposition period significantly (Table 3.5).

In the third-generation experiment, there was no effect of larval phase on the oviposition period.

Table 3.5 Effect of larval phase and adult diet on weight-related oviposition period of female *Spodoptera exempta* fed either 10% sucrose or distilled water, using first-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	3.5 ± 0.12 a (96)	3.5 ± 0.16 a (69)
Sucrose	4.9 ± 0.15 b (96)	5.0 ± 0.22 b (70)
	Phase effect: $F_{1,294}$ = 0.01 ^{ns}	
	Diet effect: $F_{1,294}$ = 98.61 ***	

Table 3.6 Effect of larval phase and adult diet on oviposition period of female *Spodoptera exempta* fed either 10% sucrose or distilled water, using third-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	4.5 ± 0.35 a (28)	4.2 ± 0.22 a (35)
Sucrose	5.7 ± 0.47 a (27)	4.9 ± 0.46 a (36)
	Phase effect: $F_{1,110}$ = 2.39 ^{ns}	
	Diet effect: $F_{1,110}$ = 7.45 **	

As Table 3.6 shows, there is a conflict between the ANOVA and Scheffe tests regarding the effect of diet on oviposition period. This is similar to what was observed on the number of egg batches (Table 3.4).

3.3.4 Pre-oviposition Period (POP)

The results for the first-generation experiment in Table 3.7 show that for both sucrose-fed and water-fed females, POP in *gregaria* was higher than in *solitaria*. However, the effect of phase is not significant in water-fed moths.

Considering the effect of adult diet, sucrose-fed *gregaria* moths had a significantly higher POP than water-fed ones. Diet had no significant effect on POP in the *solitaria* (Table 3.7).

In the third-generation experiment, larval phase did not affect POP, regardless of adult diet (Table 3.8). The ANOVA shows an overall significant effect of adult diet on the POP, but the pairwise comparisons show no differences within phases.

3.3.5) Longevity

In both first-generation and third-generation experiments, there was no significant effect of larval phase on longevity (Tables 3.9, 3.10).

The effect of adult diet shows clearly that lifespan was increased by sucrose in *solitaria* and *gregaria* in both experiments.

Table 3.7 Effect of larval phase and adult diet on pre-oviposition period of female *Spodoptera exempta* fed either 10% sucrose solution or distilled water, using first-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	2.9 ± 0.09 a (96)	3.3 ± 0.14 a (68)
Sucrose	3.1 ± 0.10 a (94)	3.6 ± 0.16 b (62)
	Phase effect: $F_{1,283}$ = 7.89 **	
	Diet effect: $F_{1,283}$ = 4.38 *	

Table 3.8 Effect of larval phase and adult diet on pre-oviposition period of female *Spodoptera exempta* fed either 10% sucrose solution or distilled water, using third-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	3.2 ± 0.23 a (28)	3.7 ± 0.19 ab (35)
Sucrose	3.7 ± 0.37 ab (27)	4.3 ± 0.35 b (35)
	Phase effect: $F_{1,109}$ = 2.59 ns	
	Diet effect: $F_{1,109}$ = 3.98 *	

Table 3.9 Effect of larval phase on weight-related longevity of female *Spodoptera exempta* fed either 10% sucrose solution or distilled water, using first-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	7.6 ± 0.48 a (74)	7.7 ± 0.49 a (52)
Sucrose	10.3 ± 0.50 b (96)	11.9 ± 0.41 b (52)
	Phase effect: $F_{1,231}$ = 0.33 ^{ns}	
	Diet effect: $F_{1,231}$ = 152.67 ***	

Table 3.10 Effect of larval phase on weight-related longevity of female *Spodoptera exempta* fed either 10% sucrose solution or distilled water, using third-generation *solitaria*. Data are presented as means ± s.e. Number of observations in parenthesis. Means followed by the same letter are not significantly different (ANOVA followed by Scheffe test).

	LARVAL PHASE	
ADULT DIET	*Solitaria*	*Gregaria*
D. Water	8.0 ± 0.39 a (27)	7.9 ± 0.26 a (32)
Sucrose	10.2 ± 0.75 b (27)	10.8 ± 0.61 b (36)
	Phase effect: $F_{1,106}$ = 0.02 ^{ns}	
	Diet effect: $F_{1,106}$ = 38.46 ***	

The ANOVA tables on which Tables 3.1 - 3.10 are based are provided in the Appendix to Chapter 3. The apparent conflict between the results of ANOVA and the Scheffe test for pairwise comparisons is discussed in Section 3.4.

3.4 DISCUSSION

The results of the experiment using first-generation *solitaria* essentially confirm the results of an earlier work (Gunn and Gatehouse, 1987) also done with first generation *solitaria*. In that study, as in this experiment, moths reared as *gregaria* in the larval stage had significantly higher fecundities than the *solitaria* moths when both phases were fed distilled water as adults. The difference in fecundity between phases is, however, less in this experiment (about 37%) than in the previous work where the *gregaria* laid about twice (a difference of 91%, calculating from the mean figures for the LK strain) as many eggs as the *solitaria* on distilled water. Also, there was no phase difference in fecundity in sucrose-fed moths in this experiment (Table 3.1) or in the previous work (Gunn and Gatehouse, 1987).

Since fecundity depends on the weight of a moth in this species (Gunn and Gatehouse, 1985, 1987) and in several other Lepidoptera (Murphy *et al.*, 1983; Hill and Pierce, 1989; Marshall, 1990), the method of correcting for weight must adequately account for the effect of this covariate. It is doubtful whether this condition is satisfied by the standard measure (number of eggs per 100 mg weight) used by Gunn and Gatehouse (1987), as it assumes a scaling constant equal to unity (i.e., a doubling of body weight results in a doubling of the level of the measured trait).

Secondly, Gunn and Gatehouse (1987) reared *solitaria* and *gregaria* larvae in different-sized containers (55ml plastic pots for *solitaria*, 500 ml Kilner jars for *gregaria*) which could present significant differences in humidity and other

environmental factors (unpublished data by K. Wilson *et al.*). The maize leaves provided for the *solitaria* larvae in the plastic pots were not kept in water as were the leaves the *gregaria* fed on. This probably affected the quality of food for the *solitaria* larvae, which effect could carry over to the adult stage and might account for part of the large difference Gunn and Gatehouse (1987) observed between water-fed *solitaria* and *gregaria* moths.

The possible influence of rearing larvae in different-sized containers and the method of correcting for weight cast some doubt on the existence of an effect of phase on fecundity as reported by Gunn and Gatehouse (1987). It is assumed that this doubt is overcome by rearing *solitaria* and *gregaria* larvae in containers of the same size in this experiment, and by the use of an analysis of covariance to correct the data for weight (see Hill and Pierce, 1989). The phase difference in fecundity must be real then, since it occurs after these modifications have been made.

Gunn and Gatehouse (1987) also found higher triglyceride levels in *gregaria* moths, but the haemolymph protein levels were comparable between the two phases. They could not find any obvious reason for the difference in weight-related fecundity, as available nitrogen has been considered the most likely factor limiting fecundity in herbivorous insects (Crawley, 1983; Strong *et al.*, 1984). Therefore it would be expected that protein (or nitrogen), rather than carbohydrate, would be limiting to egg production. It is known that some insects require carbohydrates as a source of energy for the utilization of proteins in egg production (Johansson, 1964; Engelmann, 1970). This appears to be the case in

Spodoptera exempta. If the *gregaria* moths have higher levels of energy reserves (Gunn and Gatehouse, 1987), then they have the advantage in egg production when moths have no access to dietary energy sources. The provision of sucrose seems to remove this advantage, the *solitaria* then having an alternative energy source to make up for the discrepancy in energy stored from larval nutrition.

Fecundity in both phases was increased by feeding on sucrose, but for the *solitaria* to produce an equal number of eggs, the increase in fecundity caused by sucrose-feeding (over the value on distilled water) must be greater than that in the *gregaria*. Provision of sucrose increased fecundity by 41% in the *solitaria* but by 25% in the *gregaria*, reducing the difference between phases to 12.2% from 26% on distilled water (Table 3.1).

With regard to the effect of adult diet on fecundity, the results here differ from those obtained by Gunn and Gatehouse (1985) who did not find a significant difference in fecundity between sucrose-fed and water-fed moths, provided that larval nutrition was adequate. A significant increase in fecundity was obtained in this experiment when moths were fed on sucrose, in spite of the fact that the larvae were fed *ad libitum*.

Leahy and Andow (1994) also reported a 'considerable' increase in fecundity due to sucrose-feeding in *Ostrinia nubilalis*, though fecundity in that species had previously been shown to be virtually unaffected by adult diet. This is an example of differences between experiments or workers on a similar subject. In their case, they identified differences in carbohydrate source and other

differences in experimental design as the possible reasons for the different results. In this experiment and the earlier work by Gunn and Gatehouse (1985), sucrose was the adult food, and at the same concentration. The only differences are the containers used (and the number of *gregaria* larvae per container) and the method of data analysis. In a later experiment, however, Gunn *et al.* (1989) recorded an increase in fecundity of unflown 'control' moths when provided sucrose instead of distilled water.

In the first-generation experiment, the effect of sucrose on fecundity seems to have been achieved by prolonging longevity and oviposition period and hence the number of egg batches produced (Tables 3.3 ,3.5, 3.9). However, unlike fecundity, the number of egg batches does not differ between phases even on distilled water. This implies that for the *solitaria* to even out the difference in fecundity on distilled water, sucrose-feeding should not just increase the number of egg batches but the batch size as well.

There was a phase effect on pre-oviposition period (POP), the *gregaria* having a significantly higher value than the *solitaria* when fed sucrose. POP was also influenced by adult diet, being increased when moths were fed sucrose. This increase is only significant in the *gregaria*. The migratory potential of a night-flying insect depends on the number of nights it travels and the distance covered (or the duration of flight) on each of those nights. In most insects, migration is believed to occur in sexually immature individuals, that is, during the pre-reproductive period (PRP) (Johnson, 1969, 1974). POP is an estimate of the PRP and thus an indicator of migratory potential (Johnson, 1974; Wilson, 1993). A

longer POP in the *gregaria* would therefore support the argument that they are better adapted for migration (Faure, 1943; Woodrow *et al.*, 1987). That sucrose in the adult diet further prolongs the POP suggests that it enables the *gregaria* moths to allocate more energy to flight, and the onset of oviposition is delayed to increase the number of nights for flight.

The results of the experiments using third-generation *solitaria* differ in some respects from the first-generation experiment. The effect of larval phase on fecundity is eliminated. Sucrose in the adult diet increased the fecundity in both phases but not significantly so in the *gregaria* (Table 3.2). Two factors account for the third-generation *solitaria* having a higher fecundity than the *gregaria* when fed sucrose. First, the difference between the phases on distilled water (significant in the first-generation experiment) has been so reduced as to be no longer significant. Secondly, as in the first-generation experiment, the fecundity in the *solitaria* was increased by a higher factor (about 46%) than in the *gregaria* (just over 3%) when provided sucrose. Thus for the *gregaria*, the difference in fecundity between diets remained non-significant, while the *solitaria* increased theirs significantly, resulting in an overall higher fecundity on sucrose. The different effect of sucrose on the two phases is reflected in a significant phase*diet interaction ($P<0.01$; see Appendix 3.2)

In the third-generation experiment, POP was unaffected by phase, but there was an overall diet effect due to marginal increases caused by sucrose-feeding in both phases.

Another difference between the two experiments is

that all the traits, except POP, showed a correlation with weight in the first-generation study. In the third-generation experiment, the only trait that showed a significant correlation with weight was fecundity. The reason for this is not immediately obvious.

Essentially similar to the results of the first-generation experiment, there was no phase difference in longevity of moths. Feeding on sucrose did increase longevity in both *solitaria* and *gregaria* moths.

The results of these experiments outlined above, indicate that moths in both phases are capable of producing viable eggs on distilled water alone, but sucrose does increase egg output. In this respect, *Spodoptera exempta* is similar to *Helicoverpa zea* (Lukefahr and Martin, 1964) and other species (see section 3.1). Gu and Danthanarayana (1990) state that in ecological studies of economically important species, the responses of reproductive and other activities to adult resource availability are of significance. An attempt is here made to relate the results of this study to the ecology of *S. exempta*.

It is expected that insects in both phases would maximise metabolic reserves for activities in adult life. The *gregaria* apparently lay down more lipid reserves because of the requirement for longer flights (Gunn and Gatehouse, 1987; Woodrow *et al.*, 1987). The amount of energy ultimately allocated to reproduction would depend on the energy costs of flight. Gunn *et al.* (1989) demonstrated a trade-off between flight and reproduction in *gregaria* moths that were flight-tested in the laboratory. This trade-off was observed only in moths that were denied access to a source of carbohydrate after flight, which would not

have the means to replenish the energy channelled into flight. The higher fecundity of the *gregaria* on distilled water is therefore a result of their larger energy reserves (Gunn and Gatehouse, 1987; 1993). In the field, if *gregaria* moths have no access to carbohydrates, their egg output should be lower than the levels recorded in the laboratory because of the energy expended on flight.

The lower fecundity of the *solitaria* is probably an adaptation for maintaining populations at low densities (Gatehouse, 1987) as their lower migratory potential will limit the dispersal range of larvae and increase the intensity of competition for larval resources. This may explain their lower fecundity on distilled water (Table 3.1). Availability of sucrose permits the allocation of more energy to reproduction. In the field, the availability of sucrose as nectar from flowers would depend on the amount of rainfall and would probably be an indicator also of the availability of larval food (Gunn and Gatehouse, 1987). Both of these factors will favour an increase in fecundity.

In both experiments, the difference between fecundity on distilled water and that on sucrose was greater in the *solitaria* than in the *gregaria* (Table 3.1, 3.2). There must be an upper limit to fecundity, which both phases are capable of attaining when there is adequate energy for egg production. It appears that when fed distilled water, the output of the *gregaria* is closer to this limit than that of the *solitaria*.

These experiments have not demonstrated clearly enough the effect on the reproductive biology of *S. exempta*, of rearing *solitaria* for a minimum of three generations. There is no evidence that the three-

generation treatment does accentuate the differences between phases, as far as the traits studied in these experiments are concerned. There is no obvious reason why the two phases did not differ in fecundity when fed on distilled water. Also, the *gregaria* did not respond to sucrose-feeding to the same extent as in the first-generation experiment (6.7% sucrose as opposed to 25%).

There is a problem in designing an experiment of this sort: that of seeking to enhance phase characteristics and, at the same time, attempting to reduce genetic differences between treatments. In the third-generation experiment, the *gregaria* were siblings of the *solitaria* which had been reared as *gregaria* only for one generation, the third. Thus the comparison is between a three-generation *solitaria* and one-generation *gregaria*. The basis of this design is the assumption that one generation is enough for the full switch from *solitaria* to the *gregaria* 'state' (Faure, 1943; Matthée, 1946). It would be necessary to further test the truth of this claim and to find out whether it applies to all characteristics. In locusts, gregariousness is increased by rearing for three or more generations (Uvarov, 1966). It is possible that the three-generation *solitaria* treatment reduces the level of expression of *gregaria* characters in moths.

The results of the two experiments presented in this chapter do agree in some respects, but it is conceded that there are some differences that have not been fully accounted for. For instance, it is difficult to explain the lack (in the third-generation experiment) of correlation between pharate adult weight and the traits, apart from fecundity, that were studied. There was also a conflict between results of

the ANOVA and Scheffe tests on the data for number of egg batches, oviposition period and POP for the third-generation experiment. The smaller sample sizes in this experiment may account for some of these differences.

Overall, the results seem to agree in essence, with previous work, in as far as the effect of larval phase goes, but not the effect of adult diet. The results of this work suggest that access to carbohydrates is not only necessary for body maintenance and for prolonging the lifespan of the adult, but does influence reproduction.

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CHAPTER 4

EFFECT OF PHASE ON FOOD UTILIZATION AND METABOLIC RESERVES IN LARVAE OF *SPODOPTERA EXEMPTA*

TABLE OF CONTENTS OF CHAPTER FOUR

4.1 INTRODUCTION

4.1.1 Insect nutritional ecology

Insects must consume and utilize food if they are to grow, develop and reproduce, food being the source of energy and nutrients for these other life activities (Slansky and Scriber, 1985; Hayes *et al.*, 1992; Slansky, 1993). The performance of a larva - its growth rate, developmental time, final body weight and probability of survival - are influenced by the amount and quality of food it consumes and by the rate of consumption. This influence can also carry over to affect adult performance. For example, if a larva suffers reduced growth, it may produce a small-sized adult with reduced fecundity (Slansky and Scriber, 1985). Fecundity in some insect species has been shown to depend, to a great extent, on the quality and quantity of larval diet (see Chapter Three).

Different lifestyles occur among insects as a result of differences in the quality, abundance, and persistence of food substances. In its broadest sense the subject of insect nutrition comprises the two processes of food consumption and utilization which link the physiological, behavioural, ecological and evolutionary aspects of insect life (Slansky and Scriber, 1985).

Much of the research devoted to the understanding of insect nutrition has been done in the context of nutritional ecology. The goal of such research is the determination of how the amount and quality of food influence performance (Hayes *et al.* 1992). Indices of nutrition are used to describe interactions between insects and various dietary constituents, such as

nutrients and allelochemicals, to compare insect performance on different host plants and in models to predict crop losses (Slansky and Scriber, 1985; Slansky and Rodriguez, 1987; Farrar *et al.*, 1989). These indices may also be of value in the development of pest management strategies. For instance, the development of host plant resistance tactics to manipulate insect pest performance requires an understanding of the relationship between the attributes of crop plants, their consumption and utilization by insect pests and subsequent performance (Slansky, 1993).

The degree of association of an insect species with a plant can be analysed by the determination of the efficiency with which the insect can utilize the plant as food. The suitability of a plant as food is thus linked to the feeding preferences of the insect species (Kogan and Cope, 1974).

Qualitative nutritional requirements of insects appear to be relatively uniform. This makes quantitative measurements of food intake and utilization important in ecological studies because diets may be similar in their ability to support growth, but patterns of utilization may be different. Apparently, nutritional differences must be sought on a quantitative level (Walbauer, 1968).

4.1.1.1 The Gravimetric method

An overall understanding of the utilization of a food requires information on the rate at which the food is eaten, how much of the food is digested and what proportion of digested food is incorporated as body substance or biomass.

A popular method of finding answers to these basic questions is the gravimetric method which was standardized and applied to insects by Waldbauer (1964, 1968). Modifications to Walbauer's formulae have been suggested by other authors (eg. Klein and Kogan, 1974; Ayres and Maclean, 1987). The gravimetric method involves the measurement of the dry weight of food consumed and excreted and changes in the biomass of larvae. Conversion factors are also obtained to estimate the initial dry weight of larvae and food provided (Waldbauer, 1968; Scriber and Slansky, 1981; Bowers *et al.*, 1991)

The indices of food consumption and utilization efficiency that are commonly measured include:

1. AD: The approximate digestibility or assimilation efficiency, the proportion of ingested food which is digested.
2. ECI: Efficiency of conversion of ingested food to biomass or the gross growth efficiency.
3. ECD: Efficiency of conversion of digested food to biomass or the net growth efficiency. This is also termed the metabolic efficiency because it estimates the metabolic cost of processing the food.
4. Rate of consumption: the amount of food consumed per instar or caterpillar over its life.
5. Rate of growth: the increase in body mass of the larva over the duration of an instar or the life of a larva.

AD, ECI and ECD are expressed as percentages (Walbauer, 1968; Slansky and Scriber, 1985; Bowers *et al.*, 1991; Slansky, 1993). Consumption and growth rates are expressed on a "per day" basis (CR or GR) or on a "per day per unit mass" basis (relative consumption rate- RCR, or relative growth rate- RGR).

The relative rates are preferred for comparative purposes (Slansky and Scriber, 1985; Slansky, 1993).

Farrar *et al.* (1989) distinguish three methods of calculating relative consumption and growth rates that have been used by different authors. These produce the indices RCRa and RGRa (Waldbauer, 1964, 1968), RCRg and RGRg (Gordon 1968; Klein and Kogan, 1974), RCRi and RGRi (Miller and Feeny, 1983; Dimock *et al.*, 1986). The three methods differ in the value of 'body weight' used to divide the amount of food consumed. For RCRa and RGRa, the arithmetic mean of the insect weight over the feeding period (in days) is used. Users of RCRg and RGRg prefer the geometric mean of the body weight because growth in insects tends to be exponential rather than linear. Calculation of RCRi and RGRi uses the initial weight, that is the weight of the insect at the beginning of the feeding period.

Waldbauer (1968) argued that RCRi is not as good an index of consumption as is RCRa because the former does not take growth into account. However, Farrar *et al.* (1989) are of the opinion that RCRi and RGRi are more appropriate indices of consumption and growth because they are independent of digestive efficiencies. They show that ECI tends to place an upper limit on values of indices that include mean body weight. In agreement with this line of argument, McCloskey and Isman (1995) use RCRi as a measure of consumption rate in *Mamestra configurata* Walker.

4.1.1.2 Effect of larval phase and age on food consumption and utilisation

Norris (1961) reported that crowded (*gregaria*) male *Schistocerca gregaria* "ate and excreted" more than isolated (*solitaria*) ones during the first ten days of adult life. There was also an interaction between rates of maturation and feeding, which reduced the difference in rates of consumption between phases or even caused the *solitaria* to have a higher feeding rate after the tenth day. However, density (phase) of the locusts did not affect the proportion of ingested food that was utilized. For the same species, Davey (1949) reported an 'indication' of a positive correlation between the food consumption per hopper and the number of hoppers per cage, implying that consumption increased with rearing density.

Brennière *et al.* (1949; cited by Waldbauer, 1968) obtained AD of 55%, 54% and 61% for isolated *gregaria*, *gregaria* in groups and *solitaria*, respectively, in fourth instar *S. gregaria*. Corresponding ECI values were 9%, 8%, 8%, while percentages for ECD were 16%, 15% and 13%, differences described by Waldbauer (1968) as only small.

According to Simmonds and Blaney (1986), 'solitary' larvae, of *Spodoptera exempta*, spent more time feeding on wheat than 'crowded' larvae. They also found 'feeding rates' (consumption rates) to be higher for 'crowded' larvae than for 'solitary' larvae. Iwao (1962) reported that 'crowded' *Mythimna* (*Leucania*) *separata* larvae consumed more food than 'isolated' larvae. The largest difference was in the 6th instar, where the crowded larvae ate 1.2 times more food than isolated larvae.

The rate of food consumption and the efficiency of its utilization is likely to differ from instar to instar or even within an instar (Walbauer, 1968; Slansky and Scriber, 1985; Slansky, 1993). The relative rate of consumption tends to be higher in the earlier instars, but for most species, the last two or three instars account for a large proportion of total lifetime consumption (Waldbauer, 1968; Scriber and Slansky, 1981; Slansky and Scriber, 1985, Slansky, 1993). *Bombyx mori* and *Protoparce sexta* (Johan.) consume about 99% of their total intake during the last three instars (Waldbauer,1968). For *Spodoptera exempta*, Brown and Odiyo (1968) recorded a consumption of 106.6 mg dry weight for a group of 12-day old larvae (instar not specified), while the same group consumed 74.36 mg in their first ten days. Differences between phases in rates of consumption or utilization efficiency may, therefore, depend on the age of the larvae. In *Mythimna separata*, no differences in food consumption were apparent between crowded and isolated larvae in the first three instars (Iwao, 1962).

4.1.2 Effect of phase and age on lipid metabolism in insects

Lipids are important sources of metabolic energy for cell maintenance, reproduction, embryogenesis and metamorphosis. They are of special importance to migrant insects because fat is the main source of energy for migratory flight (Macaulay, 1974; Hoppe *et al.*, 1975; Jackson and Armold, 1977). The lipid content of an insect may determine its longevity. For instance, female mosquitoes supported by lipids can survive for a 'long time' in spite of unfavourable nutritional and climatic conditions (Van Handel,

1985).

In *Autographa* (*Plusia*) *gamma* the mean percentage fat content of larvae was higher for crowded than for solitary larvae. The difference in water content was, however, not significant (Long, 1953). In the same study, fat content was negatively correlated with weight in solitary larvae, but positively correlated in crowded larvae. The reverse relation was found between water content and body weight: positive in solitary but negative in crowded though the correlation in the crowded larvae was not significant. Macaulay (1974) also found a positive correlation between dry weight and fat content of *A. gamma* larvae. It is not clear from Macaulay's paper whether the larvae referred to were reared solitary or in groups, but these findings show that it is necessary to consider the effect of body weight in comparing lipid contents.

Matthée (1945) compared amounts of larval body fat between *solitaria* and *gregaria* larvae in the final instar in *Spodoptera exempta* and *S. abyssinia*. *Gregaria* larvae had a higher fat content. Similarly, fifth instar *gregaria* hoppers had a higher fat content than *solitaria* hoppers in the same instar in both *Locusta migratoria* and *Locustana pardalina*. However Matthée found no difference in water content between the phases in *S. abyssinia* or *Locusta migratoria*. Gunn and Gatehouse (1987) reported that the abdominal triglyceride content of *Spodoptera exempta* at emergence, was 2.5 to 6.1 times greater in *gregaria* than *solitaria* moths.

Macaulay (1974) observed that fat accumulated steadily in the larval stage of *Autographa gamma* up to a maximum of 20 mg, about 20% of the dry weight of the

insect. This proportion was reduced during the pupal stage to about 10% in the pharate adult. Laughlin (1956) found, in *Phyllopertha horticola* (L.), that half the fat reserves accumulated during larval life had been used by the time the adult emerged. These results point to a relation between the lipid content and the age of insects at various stages of development.

Over a third of the total loss in dry weight during embryogenesis in *Spodoptera exigua* is a result of lipid depletion which in turn is primarily due to triglyceride metabolism (Hoppe *et al.*, 1975). Van Handel (1985) also found that in adult *Aedes aegypti* the variability of lipid content is largely due to variability of triglycerides. In adult *Spodoptera exempta*, lipid reserves are held principally as triglycerides in the abdomen (Gunn and Gatehouse, 1987). Triglyceride levels therefore seem to be a good indicator of differences in lipid content of insects subjected to different experimental treatments.

4.1.3 Objectives of study

1. To assess the extent to which food consumption and utilization in larvae of *Spodoptera exempta* are affected by the phase polyphenism and whether any phase effects are related to the age (instar) of the larvae.

2. To study the effects of phase on triglyceride levels in larvae of *S. exempta*, how they change with instar and to relate any changes with the reported higher levels of triglyceride in *gregaria* moths (Gunn and Gatehouse, 1987).

The effect of rearing containers on triglyceride and

water contents of larvae will also be investigated.

4.2 MATERIALS AND METHODS

4.2.1 Effect of phase and age on food consumption and utilization by *S. exempta* larvae

This experiment was carried out in two parts, one using first generation *solitaria* and the second, third generation *solitaria* as defined in Chapter Two. In the first generation experiment, *solitaria* were reared individually in 120 ml glass jars while *gregaria* were reared in groups of 8 in 500 ml Kilner jars. The temperature in the culture rooms during this experiment was 28 ± 2°C, and relative humidity at 65 ± 5% and 12L:12D.

Larvae were fed fresh maize leaves daily, which were weighed before being given to the larvae. Left-over food was separated from faeces and both dried in an oven at 80°C to constant weight. Initial dry weight of food provided was estimated from the percentage dry matter content of aliquots of maize leaves which were also dried in the oven to constant weight.

Data on daily food consumption, frass production and larval weight gain were collected from the beginning of the fourth to the end of the sixth instar. Four *solitaria* and four *gregaria* larvae were sacrificed at the beginning and end of each instar for freeze drying. The percentage dry matter of these samples was used in estimating the dry weight of the larvae, so that nutritional indices could be calculated on a dry weight basis. Larvae from two families were used for the first-generation study.

The experiment using third generation larvae was done under the same laboratory conditions and following the same procedure for data collection. The

only difference was in the rearing of the *gregaria* in groups of four in 120 ml containers. Only one family was studied in this experiment. Mean values of five nutritional indices - AD, ECI, ECD, RCRi and RGRi - were calculated for fourth to sixth instar larvae in each phase after Waldbauer (1968), Slansky and Scriber (1985) and Farrar *et al*. (1989) as follows:

$$AD = 100\ (I-F)/I$$

$$ECI = 100\ P/I$$

$$ECD = 100\ P/(I-F)$$

$$RCRi = I/(Bi)(T)$$

$$RGRi = P/(Bi)(T),$$

where Bi = initial weight of the larva

F = dry weight (mg) of faeces produced

I = dry weight (mg) of food eaten

P = larval mass gained (mg dry weight) over time period T (days).

Groups of *solitaria* (8 per group, first-generation; 4 per group, third-generation) larvae were chosen at random and mean values of the various indices calculated for them over each of the three instars. This provided units that were comparable to the replicates of the *gregaria* treatment for analysis. This grouping was necessary because it was not possible to collect data on a 'per larva' basis in the *gregaria*. A repeated measures analysis of variance (ANOVA) was then carried out on the data to measure the effect of larval phase and instar on each of the nutritional indices or growth rates.

4.2.2 Effect of phase and age on triglyceride and water contents of larvae

Larvae were reared *solitaria* (1 per 120 ml glass jar) or *gregaria* (4 per 120 ml glass jar) as described in Chapter Two. Only third-generation *solitaria* were used, with larvae from two families. A sample of larvae were taken out of the culture at the end of the fourth, fifth and sixth instars for triglyceride analysis. The end of each instar was marked by the beginning of the quiescent, non-feeding stage.

The larvae to be used for triglyceride analysis were weighed, frozen at -70°C for 24 hours, freeze-dried and then re-weighed. The difference in fresh and dry weights was recorded as the water content of the larvae. The larvae were then analysed for triglycerides using an adaptation of the method used by Eggstein and Kuhlman (1974) which was used on pharate adult *Spodoptera exempta* by Gunn and Gatehouse (1987). This method is summarised in Appendix 4.1. Reagents from the Sigma quantitative semi-enzymatic triglyceride kit were used for the assay.

An analysis of covariance was done on the data for water and triglyceride content to account for any differences in weight between phases. Owing to differences in variability of triglyceride levels between the two families studied, the data were analysed separately.

4.2.2.1 Effect of rearing containers on the triglyceride and water contents of larvae

Larvae were reared as *solitaria* (third generation) in either a 55 ml plastic pot or a 120 ml glass jar.

Gregaria larvae, obtained from the same batch of eggs as the *solitaria*, were reared in groups of four in 120 ml glass jars or in groups of 8 in 500 ml Kilner jars. In the plastic pots, the larvae fed on maize leaves that were not provided with water. All the other containers had vials of water for the larval food. At the end of the sixth instar, larvae from the various treatments were weighed, frozen and freeze-dried and re-weighed to determine their water contents. Triglyceride levels were then measured as described in section 4.2.2.

4.3 RESULTS

4.3.1 Effect of phase and age on food consumption and utilization by *S. exempta* larvae

In tables 4.1-4.6, 'P' indicates the level of significance of the F ratios from the ANOVA or ANCOVA using these symbols:

* = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$,

*** = $P \leq 0.001$, ns = not significant.

Table 4.1 Effect of phase and age on food utilization efficiency over three instars in larvae of *Spodoptera exempta*, using first-generation *solitaria*. Results are expressed as means ± se. Means within a phase or instar that are followed by the same letter are not significantly different (ANOVA followed by Bonferroni test).

INDEX/INSTAR	*Solitaria*	*Gregaria*
A.D.(%)		
4th	69.3 ± 4.72a	65.5 ± 12.1a
5th	58.1 ± 3.89ab	51.4 ± 3.39ab
6th	43.4 ± 3.21b	43.5 ± 4.92b
E.C.I.(%)		
4th	11.6 ± 1.21a	11.9 ± 2.96ac
5th	21.0 ± 0.51b	19.6 ± 1.87ab
6th	8.8 ± 0.69ac	5.0 ± 0.40c
E.C.D.(%)		
4th	18.9 ± 3.17a	27.3 ± 10.2ab
5th	37.2 ± 2.35a	38.0 ± 2.29a
6th	21.6 ± 3.42ab	11.9 ± 1.23b

	Phase effect	P	Instar effect	P
AD:	$F_{1,16}$ = 0.28	ns	$F_{2,16}$ = 11.13	***
ECI:	$F_{1,16}$ = 12.12	**	$F_{2,16}$ = 26.22	***
ECD:	$F_{1,16}$ = 0.00	ns	$F_{2,16}$ = 8.36	**

n = 5 (groups of 8 larvae)

Figure 4.1 Effect of phase and age on larval food utilization efficiency in *S. exempta*, using first-generation *solitaria*.

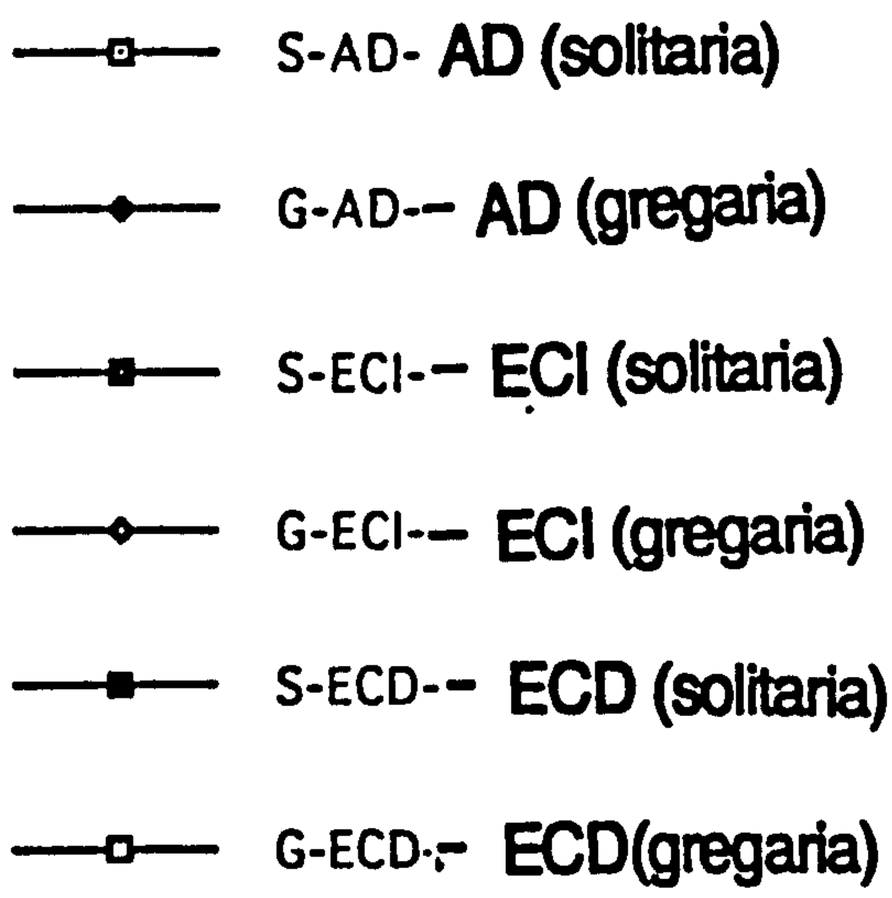

Figure 4.1

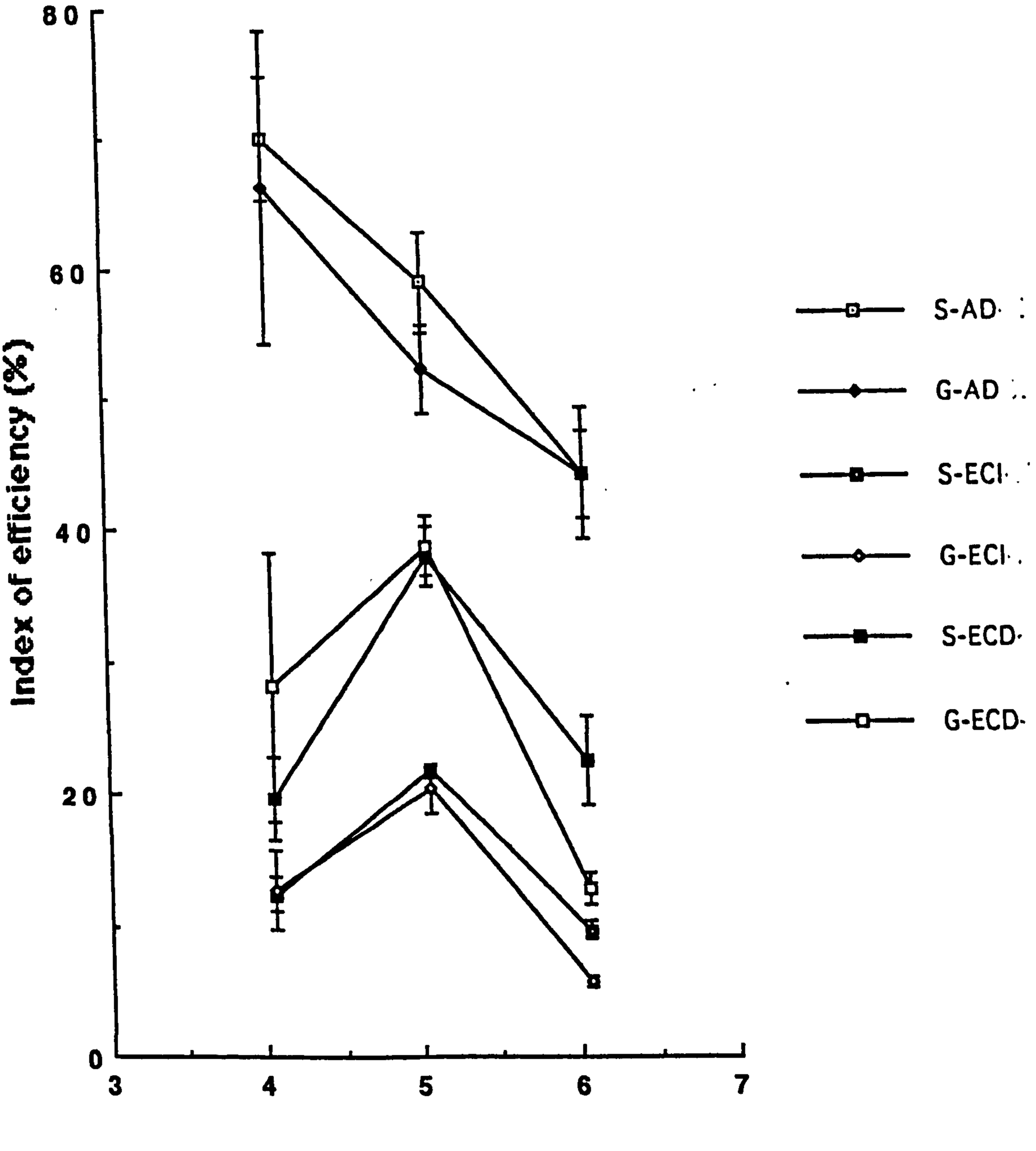
80
60
40
20
0
Index of efficiency (%)
3
4
5
6
7
Larval instar (Ist generation)
S-AD
G-AD
S-ECI
G-ECI
S-ECD
G-ECD

Table 4.2 Effect of phase and age on rates of food consumption (RCRi) and growth (RGRi) over three instars in larvae of *Spodoptera exempta*, using first-generation *solitaria*. Results are expressed as means ± se. Means within a phase or instar that are followed by the same letter are not significantly different (ANOVA followed by Bonferroni test).

RATE /INSTAR	*Solitaria*	*Gregaria*
RCRi (mg/mg/day)		
4th	26.4 ± 2.50a	20.0 ± 3.76a
5th	12.0 ± 0.58b	10.2 ± 1.69b
6th	14.9 ± 1.57b	10.3 ± 3.19b
RGRi (mg/mg/day)		
4th	2.9 ± 0.34a	2.6 ± 1.02a
5th	2.5 ± 0.13a	2.1 ± 0.33a
6th	1.2 ± 0.10a	0.5 ± 0.17a

	Phase effect	P	Instar effect	P
RCRi:	$F_{1,16}$ = 6.21	*	$F_{2,16}$ = 12.61	***
RGRi:	$F_{1,16}$ = 2.65	ns	$F_{2,16}$ = 7.16	**

n = 5 (groups of 8 larvae)

Tables 4.1 and 4.2 show the indices of food utilisation and growth in first-generation *solitaria* and *gregaria* larvae. Figures 4.1 and 4.2 summarise the changes in AD, ECI, and ECD for larvae in the first- and third-generation experiments respectively.

The trend of changes in the nutritional indices from the fourth to sixth instar was similar for the two phases. Approximate digestibility (AD) decreased from the fourth to the sixth, but only the difference between the fourth and sixth instars was significant. There was no difference between phases in any of the

three instars.

The efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) both increased from the fourth to fifth instar but fell between the fifth and sixth. In the *solitaria*, ECI was significantly higher in the fifth than the fourth or sixth instar. In the *gregaria* larvae, the only significant difference in ECI was between the fifth and sixth instars. (Table 4.1). There were no phase differences within instars, but the combined mean ECI for all *solitaria* (13.8%) was significantly higher than that for the *gregaria* (12.1%) (P= 0.008). Within phases, the only significant difference in ECD was between fifth- and sixth-instar *gregaria*. ECD did not differ between phases.

Consumption rate (RCRi) was higher in the *solitaria* than in the *gregaria* larvae (Table 4.2). In both phases, the rate was highest in the fourth instar falling by 49 to 54.5% in the fifth instar, but rising slightly again in the sixth.

Similar data obtained in the experiment using third- generation *solitaria* are presented in Table 4.3. Like the first-generation experiment, AD in both phases was highest in the fourth instar and decreased with increasing age. In addition to the effect of instar, there was a significant phase effect, the *solitaria* having a higher assimilation efficiency. In the sixth instar, however, there was no significant difference between phases.

ECI and ECD values were higher for the *gregaria* in the third-generation experiment, except in the sixth instar, where the differences between phases were not significant. In both phases, ECI and ECD were

highest in the fifth instar. While ECD was lowest in the fourth instar in the *solitaria*, in the *gregaria*, it was lowest in the sixth instar. The inter-instar differences were, however, not significant in the *solitaria* (Table 4.3).

RCRi fell from fourth to sixth instar in the *solitaria*, but in the *gregaria*, it was lower in the fifth than the fourth and sixth instars. Within instars, the only significant phase difference was in the fourth instar, where the *solitaria* had a higher rate of consumption than the *gregaria*. Within phases, consumption rate in sixth instar *solitaria* was significantly lower than the rate in the fourth instar.

There was no difference between phases in rate of growth (RGRi), but as in RCRi, the inter-instar changes were different for the two phases. In the *gregaria*, growth rate fell consistently from fourth to sixth instar (though not significantly) but in the *solitaria*, the fifth instar has the highest value, significantly higher than the rate of growth in the sixth instar (Table 4.4).

Table 4.3 Effect of phase on food utilization efficiency over three instars in larvae of *Spodoptera exempta*, using third-generation *solitaria*. Results are expressed as means ± se. Means within a phase or instar that are followed by the same letter are not significantly different (ANOVA followed by Bonferroni test).

INDEX/INSTAR	*Solitaria*	*Gregaria*
A.D.(%)		
4th	63.6 ± 2.77a	46.1 ± 5.01c
5th	55.2 ± 3.03ab	38.7 ± 1.49c
6th	45.3 ± 2.30b	32.4 ± 1.95bc
E.C.I.(%)		
4th	11.0 ± 0.64a	20.7 ± 1.41bc
5th	22.8 ± 1.33b	25.2 ± 1.15b
6th	18.5 ± 0.76bc	16.2 ± 0.88c
E.C.D.(%)		
4th	24.4 ± 3.35a	50.3 ± 7.72bc
5th	44.2 ± 4.63a	66.3 ± 4.75c
6th	36.2 ± 3.01a	43.7 ± 4.67ab

	Phase effect	P	Instar effect	P
AD:	$F_{1,28}$ = 80.68	***	$F_{2,28}$ = 11.62	***
ECI:	$F_{1,28}$ = 21.16	***	$F_{2,28}$ = 28.48	***
ECD:	$F_{1,28}$ = 31.43	***	$F_{2,28}$ = 6.65	**

n = 8 (groups of 4 larvae)

Figure 4.2 Effect of phase and age on larval food utilization efficiency in *S. exempta*, using third-generation *solitaria*.

S-AD- AD (solitaria)

G-AD-- AD (gregaria)

S-ECI-- ECI (solitaria)

G-ECI-- ECI (gregaria)

S-ECD-- ECD (solitaria)

G-ECD-- ECD(gregaria)

Figure 4.2

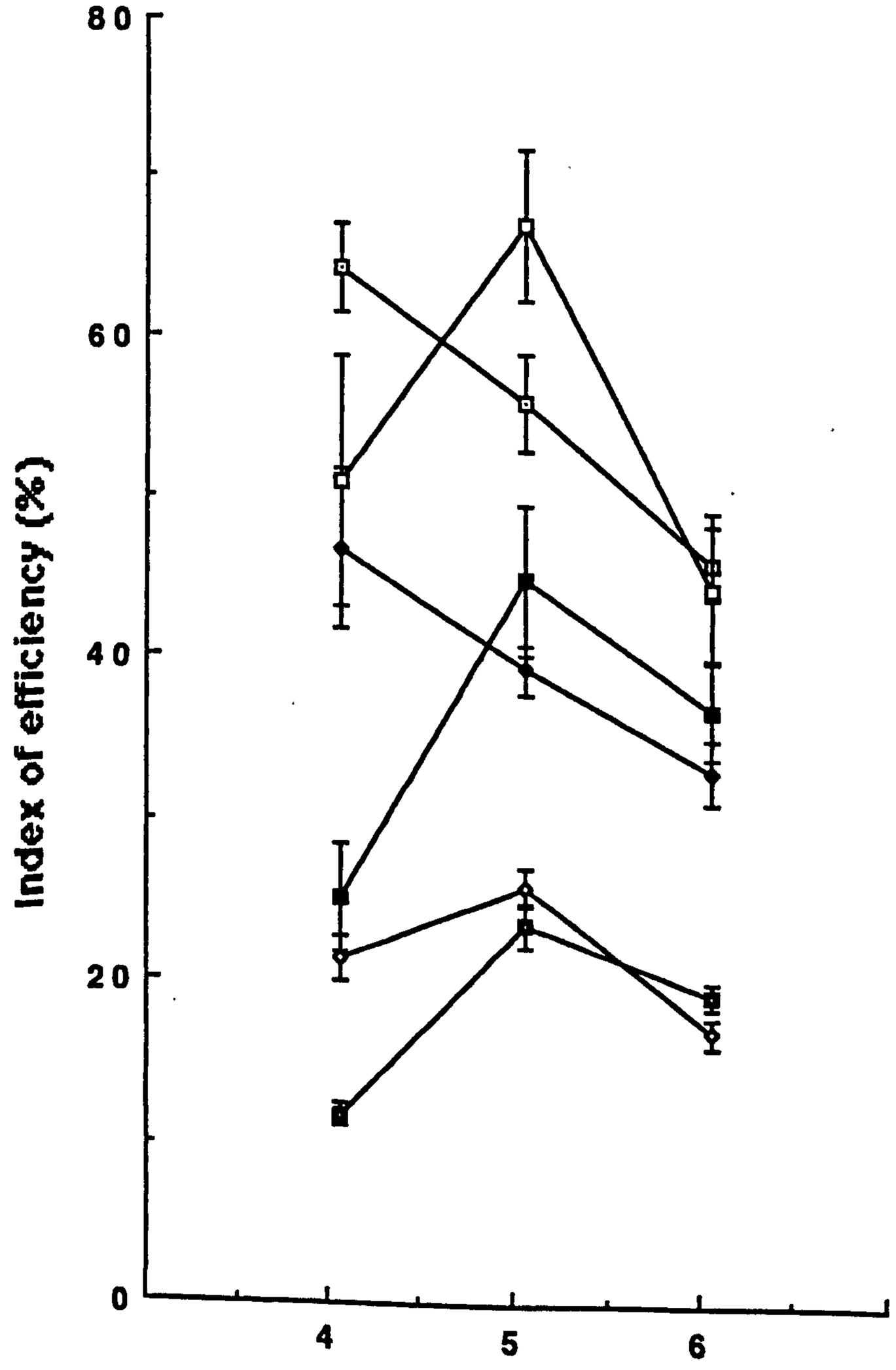
80
60
40
20
0
Index of efficiency (%)
4
5
6
Larval instar (3rd generation)

Table 4.4 Effect of phase and age on rates of food consumption (RCRi) and growth (RGRi) over three instars in larvae of *Spodoptera exempta*, using third-generation *solitaria*. Results are expressed as means ± se. Means within a phase or instar that are followed by the same letter are not significantly different (ANOVA followed by Bonferroni test).

RATE /INSTAR	*Solitaria*	*Gregaria*
RCRi (mg/mg/day)		
4th	11.7 ± 1.13a	8.5 ± 0.91b
5th	8.9 ± 0.79ab	6.1 ± 0.85b
6th	7.1 ± 0.23b	8.3 ± 0.33b
RGRi (mg/mg/day)		
4th	1.45 ± 0.08ab	1.78 ± 0.14a
5th	1.88 ± 0.15b	1.55 ± 0.22ab
6th	1.38 ± 0.06a	1.41 ± 0.07a

	Phase effect	P	Instar effect	P
RCRi:	$F_{1,28}$ = 4.74	*	$F_{2,28}$ = 8.14	**
RGRi:	$F_{1,28}$ = 0.00	ns	$F_{2,28}$ = 3.81	*

n = 8 (groups of 4 larvae)

4.3.2 Effect of phase and age on triglyceride and water contents of larvae

Mean changes in total triglyceride and water contents of *gregaria* and *solitaria* larvae from the fourth to the sixth instar are presented in Tables 4.5 and 4.6 for families 1 and 2 respectively.

Table 4.5 Effect of phase on total triglyceride or water content over three instars in larvae of *Spodoptera exempta*, using third-generation *solitaria* (Family 1).. Results are expressed as means ± se; means are adjusted for weight. Number of observations in parentheses. Means within a phase or instar that are followed by the same letter are not significantly different (ANOVA followed by Bonferroni test).

TRIGLYCERIDE / WATER CONTENT		
INSTAR	*Solitaria*	*Gregaria*
Triglyceride (mg/dl)		
4th	190.9 ± 1.42a (9)	189.2 ± 1.14a (11)
5th	158.2 ± 2.33a (10)	164.4 ± 2.31ac (12)
6th	81.1 ± 20.5b (10)	132.5 ± 13.7c (10)
Water (% body weight)		
4th	84.8 ± 0.62a (9)	83.9 ± 0.20a (11)
5th	86.4 ± 0.19a (10)	85.9 ± 0.24a (12)
6th	84.5 ± 1.36a (9)	79.7 ± 1.26b (10)

	Phase effect	P	Instar effect	P
Triglyceride:	$F_{1,54} = 5.98$	*	$F_{2,54} = 11.08$	*
Water :	$F_{1,54} = 8.24$	**	$F_{2,54} = 11.21$	**

Table 4.6 Effect of phase on total triglyceride or water content over three instars in larvae of *Spodoptera exempta*, using third-generation *solitaria* (Family 2). Results are expressed as means ± se; means are adjusted for weight. Number of observations in parentheses. Means within a phase or instar that are followed by the same letter are not significantly different (ANOVA followed by Bonferroni test)

	TRIGLYCERIDE / WATER CONTENT	
INSTAR	*Solitaria*	*Gregaria*
Triglyceride (mg/dl)		
4th	169.8 ± 4.26a (11)	166.8 ± 3.20ac (13)
5th	145.6 ± 4.10a (11)	141.8 ± 4.57a (10)
6th	278.7 ± 17.6b (10)	195.0 ± 12.4c (14)
Water (% body weight)		
4th	86.4 ± 0.28a (11)	86.1 ± 0.29a (13)
5th	87.1 ± 0.47a (11)	86.9 ± 0.22a (10)
6th	78.5 ± 1.92b (10)	73.0 ± 0.96c (14)

	Phase effect	P	Instar effect	P
Triglyceride:	$F_{1,62}$ = 10.25	**	$F_{2,62}$ = 11.08	***
Water :	$F_{1,62}$ = 16.30	***	$F_{2,62}$ = 11.21	***

In both families, there were significant effects of phase and instar on both triglyceride and water contents. There were also significant phase-instar interactions. In Family 1, the weight-adjusted triglyceride contents were highest in the fourth instar, reducing to the lowest levels in the sixth. The two phases had equal amounts of lipid in the

fourth instar, but the *gregaria* began to show higher levels in the fifth. By the sixth instar, the *gregaria* had 1.6 times more triglyceride than the *solitaria*, which is a significant difference ($P < 0.5$). Corresponding to the differences in triglyceride levels, the *solitaria* contain more water in all three instars. The difference is greatest (4.8 %)and only significant in the sixth. Unlike the continuous reduction in triglyceride levels with age, the water content rises in the fifth instar in both phases.

Family 2 presents a different pattern (Table 4.6) from Family 1. Water content is again higher in the *solitaria*, significantly so in the sixth instar (5.5% higher in *solitaria*). Like in family 1, there is a small rise in water level (0.7% in *solitaria*, 0.8% in *gregaria*) between the fourth and fifth instars, and a reduction in the sixth. The striking difference between families is in the triglyceride content. First, the levels in Family 2 are lowest in the fifth instar, and increase dramatically in the sixth. Then there is a reversal of the phase difference, the *solitaria* having higher levels of triglyceride. In the sixth instar, the difference is as high as 42.9%. This trend and the high value in the sixth instar look odd.

4.3.2.1 Effect of rearing containers on triglyceride and water contents

Table 4.7 Effect of size of rearing container on water and triglyceride content of *S. exempta* in the sixth instar. *Solitaria* were reared in 55 ml or 120 ml containers; *gregaria* at 4 per 120ml or 500ml container. Results are expressed as means ± se; means are adjusted for weight. Number of observations in parenthesis.

	TRIGLYCERIDE (Tgl) / WATER CONTENT	
CONTAINER	*Solitaria*	*Gregaria*
Triglyceride (mg/dl)		
55 ml	309.0 ± 10.8 (23)	-
120 ml	276.2 ± 10.5 (24)	361.6 ± 14.2 (26)
500 ml	-	359.1 ± 16.4 (23)
Water (% body weight)		
55 ml	68.4 ± 1.01 (23)	-
120 ml	70.7 ± 0.29 (24)	69.1 ± 0.34 (26)
500 ml	-	68.5 ± 0.43 (23)

Container effect

Triglycerides : P = 0.000
Water : P = 0.021

Contrasts

Triglyceride : sol 55ml = sol 120ml < gre 120ml = gre 500ml
Water : sol 120ml > sol 55ml = gre 120ml = gre 500ml

Table 4.7 presents the data on the water and triglyceride contents of *solitaria* and *gregaria* larvae reared in containers of different sizes. *Solitaria*

larvae reared in 120 ml glass jars had the highest water content and the lowest level of triglycerides among all treatments. *Solitaria* in 55 ml plastic containers had a lower water content and higher triglyceride content than those in 120 ml glass jars but the difference in triglycerides was not significant.

Gregaria reared in the two sizes of glass jars did not differ in water or triglyceride content. *Solitaria* in the 55 ml containers had the same water content as the *gregaria*, but they contained significantly less triglycerides.

4.4 DISCUSSION

All nutritional indices measured in both experiments show significant effects of instar (Tables 4.1-4.4). Approximate digestibility (AD) decreases with instar. This agrees with the general trend among insect species for which this index has been measured (Waldbauer, 1968; Slansky, 1985). Among reasons offered for the reduction of AD with age is that older larvae may be less selective in feeding, consuming a higher proportion of indigestible fibre (Kogan and Cope, 1974). However, even where the food quality remains constant, AD may still change with age (Chaplin and Chaplin, 1981). Another factor that is thought to be involved is changes in the ratio of gut surface to body size. An animal that doubles its weight and volume will only increase the surface area of its digestive tract by a factor of 1.8. Also, smaller chewing insects chew off smaller pieces of food and thus present a greater surface area for digestion (Waldbauer, 1968; Slansky and Scriber, 1985).

In both experiments in this study, (first, using first-generation *solitaria*; second, using third-generation *solitaria*), ECI (efficiency of conversion of ingested food) and ECD (efficiency of conversion of digested food) are higher in the fifth than in the fourth and sixth instars (Tables 4.1, 4.3). Reviews by Walbauer (1968) and Slansky and Scriber (1985) show that ECD generally increases with age while ECI increases, decreases or remains unchanged depending on the interaction between AD and ECD. However, patterns appear to vary with species. Of 26 species for which Slansky and Scriber (1985) compared ECD values between

late and early instars, 5 species had a higher value in the early instars. The 5 species include one lepidopteran, *Pseudoplusia includens* for which Kogan and Cope (1974) obtained 50, 57 and 41% ECD for the fourth, fifth and sixth instars respectively. It appears that the fifth instar larva of *Spodoptera exempta* is more efficient at conversion of assimilated food to biomass than the fourth or sixth. It is not obvious why this is the case.

Neither AD nor ECD was affected by phase in the first experiment (Table 4.1). For ECD, this must be due, at least in part, to the high variability of the data, especially in the fourth instar *gregaria*. The variability is probably the result of differences in feeding behaviour and development rates among individuals forming the groups of *gregaria* larvae. Such differences must be greater the larger the size of the group, hence the decision to reduce the group size in the third-generation experiment.

In the third-generation experiment, variability was lower than it was in the first-generation experiment which probably helped to bring out the differences between phases. *Solitaria* have a higher AD in all three instars (Table 4.3). ECI is higher for the *gregaria* in the fourth and fifth instars, but in the sixth, the value for the *solitaria* is higher, which is also the case in the first-generation experiment (Table 4.1). The greater conversion efficiency of the *gregaria* shows in consistently higher values of ECD in all instars, though the difference is not significant in the sixth instar.

Solitaria larvae have higher consumption (RCRi) rates in the first-generation experiment (Table 4.2). In the second experiment (third-generation *solitaria*),

the *solitaria* again have higher RCRi except in the sixth instar when the *gregaria* consume more than the *solitaria* (Table 4.4). According to Slansky and Scriber (1985), the high end of the range of RCR values is 3 - 6 mg mg^{-1} d^{-1}, but values higher than 20 mg mg^{-1} d^{-1} were obtained in this study. Slansky and Scriber's values must be for RCRa while RCRi is used here, the latter not being limited by the values of digestive efficiencies (Farrar *et al.*, 1989). Calculation of RCRa for the two experiments yields 3.5-8.9 mg mg^{-1} d^{-1} (*solitaria*), 4.0-8.7 mg mg^{-1} d^{-1} (*gregaria*) in the first experiment and 2.6-4.6 mg mg^{-1} d^{-1} (*solitaria*), 2.3-3.7 mg mg^{-1} d^{-1} (*gregaria*) in the second experiment. These values are closer to the range given by Slansky and Scriber (1985).

The higher rates of consumption for the *solitaria* larvae seem to contradict the findings of Simmonds and Blaney (1986) who reported higher feeding rates (mg/min) for the *gregaria*. However, their study was probably limited to the sixth instar only in which case it is similar to the result of the second experiment (Table 4.4). Again, unless Simmonds and Blaney (1986) measured the feeding rates through the entire duration of the instar(s), the values may not be representative, because rates do vary even within instars (Waldbauer, 1968; Slansky, 1993).

The phase differences in food consumption or efficiency of utilization are not reflected in rates of growth in either experiment (Tables 4.2 and 4.4), which is rather surprising. Chaplin and Chaplin (1981) compared assimilation efficiency of nymphs of the migratory milkweed bug, *Oncopeltus fasciastus* with that of the non-migratory milkweed bug, *Lygaeus kalmii* on milkweed seed. They concluded that the migrant grew

more rapidly than the resident by maintaining a higher assimilation efficiency. In *Spodoptera exempta*, both phases migrate, but previous evidence on duration of tethered flights (Woodrow *et al.*, 1987) and metabolic reserves (Gunn and Gatehouse, 1987) suggest that the *gregaria* phase has a greater migratory potential. It is to be expected, on this basis, that the *gregaria* will have higher conversion efficiencies to support a higher rate of growth and development, and storage of energy for longer flights. It is difficult to explain why the observed differences in utilization efficiency between the two phases are not translated into differences in the rates of growth.

There are some differences between the results of first-generation and third-generation experiments. Notably, in the first experiment, the only phase difference among the three indices of food utilization was in ECI, the *solitaria* having the higher value. In the second experiment, phase had an effect on all three indices, the *gregaria* having higher ECI and ECD while the *solitaria* had higher AD.

The magnitude of AD between experiments is about the same for the *solitaria* (43.4-69.3%, Table 4.1 and 45.3-63.6%, Table 4.3), while for the *gregaria* they are lower in the third-generation experiment (32.4-46.1 %, Table 4.3) than in the first-generation experiment (43.5-65.5%, Table 4.1). Again from Figures 4.1 and 4.2, ECD's are higher for both phases, while the values of ECI are of about the same order. The higher ECD's indicate a higher efficiency of assimilation of food. This must be related to the fact that RCRi's are lower in the third-generation experiment than in the first-generation experiment (Tables 4.2, 4.4). The lower rates of consumption

probably permit more efficient utilization but, in spite of this, RGRi's were higher in the first-generation experiment.

It is difficult to attribute any of these differences to an effect of the number of generations the *solitaria* have been so reared. This cannot be fully considered because different group sizes were used for the *gregaria* in the two experiments.

There are significant effects of both phase and instar on triglyceride levels in the two families studied (Table 4.5, 4.6). In Family 1, there is no difference between phases in the fourth and fifth instars. In the sixth, *gregaria* have significantly more triglycerides than *solitaria* (1:1.6). The *gregaria* also contain significantly less body water than the *solitaria* in the sixth instar. This result supports Gunn and Gatehouse's (1987) finding that *gregaria* pharate adults contained more triglyceride than *solitaria*. That the difference in lipid levels between the phases was only significant in the sixth instar may be related to the fact that the phase change (at least in its manifestation in change of skin colour) occurs after the third instar. The phases might be expected to get more divergent in physiology in the later instars.

There is a reversal of the phase effect in Family 2 as well as the change in lipid levels with age. As in Family 1, it is in the sixth instar that the difference in lipid gets significant, but the *solitaria* have more lipid than the *gregaria*. While in Family 1, the weight-adjusted means decrease from 4th to 6th instar, in F2, they decrease in the 5th instar but rise again sharply in the 6th in both phases. The water levels follow similar trends in both families.

They are higher in the *solitaria*, tend to increase slightly from 4th to 5th instar, and fall in the 6th. In spite of the higher lipid levels in the *solitaria* in Family 2, the water content remains higher than that of the *gregaria* in the 6th instar. This is also difficult to explain because overall, there is a significant negative correlation between water and triglycerides (Family 1, r^2 = 0.81; Family 2, r^2 = 0.96) in both families.

The extremely high value for triglyceride in the sixth-instar *solitaria* in Family 2 is puzzling. The difference in biochemistry between the two families can hardly be attributed to different environmental conditions as they were both reared in the same laboratory over the same period and under similar conditions.

Whether the higher water levels in the fifth instar are related to the higher food conversion efficiency in the same instar is not clear and requires further investigation.

The results of the experiment on container effect (Table 4.7) indicate that within-phase triglyceride levels are not affected by the type of container in which larvae are reared. The P value under container effect (Table 4.7) is actually the effect of phase, as the containers with *gregaria* produced significantly higher levels of lipid. Water levels in the *gregaria* were not affected by container type either. However, *solitaria* larvae reared in 120 ml glass jars contained significantly more (2.3%) water than those in the 55 ml plastic containers. A possible reason for this is that the larvae in the plastic containers did not have pots of water for the maize leaves, as did all the glass containers. Records of the colour of the larvae

also show that while all the *solitaria* larvae in glass jars were green or brown (typical *solitaria* colours) about 40% of those in the plastic pots were dark green (closer to the *gregaria* end of the spectrum). The reason for this colour difference is not certain, but it might have contributed to the difference of 32.8 mg/dl between the means of the two treatments which is rather high, though not significant.

In conclusion, differences have been found between phases in food consumption and utilization and in the storage of metabolic reserves but certain contradictions make the findings difficult to interprete in terms of the life-history strategies of the species. It is also difficult to establish a link between the two aspects of nutrition and storage of energy. Perhaps other environmental and genetic factors also need to be considered for a clearer picture to emerge.

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CHAPTER FIVE

THE EFFECT OF LARVAL PHASE ON THE THERMAL ECOLOGY OF *SPODOPTERA EXEMPTA*

TABLE OF CONTENTS OF CHAPTER FIVE

5.1 INTRODUCTION

5.1.1 Temperature and Insect Development

The importance of environmental temperature in insect development is widely recognised. McDonald (1990) describes temperature as the driving force among the factors that regulate development rates in insects. Generally, temperature is considered most important in its effects on rates of development and growth of ectotherms (Begon *et al.*, 1990).

The management of insect pests usually involves the forecasting of events in the life history of the insect. Such predictions require knowledge of the development of the insect and they are often based on the thermal requirements of the species. This, again, reflects the importance of temperature in the development process (Wagner *et al.*, 1984; McDonald, 1990).

One method for modelling the temperature-time relationship in insect development is the use of thermal summation or day-degrees. This is based on the idea of 'physiological time' which implies that the rate at which time passes for a developing insect depends on the temperature regime it experiences (Taylor, 1981; Begon *et al.*, 1990). Accumulated degree days are estimated by first determining the minimum temperature at which development occurs (the threshold temperature). Degree days accumulate when temperatures are above this threshold. Wagner *et al.* (1984) point out certain limitations of this method, notably the fact that the threshold temperature is determined empirically rather than experimentally, and suggest an alternative method. In spite of this, the 'day-degree'

method is still widely used for predicting insect development times (Roberts and Mahr, 1986; Miller and Maddox, 1991; Spivak *et al.*, 1992; Miller, 1992; Barker and Enz, 1993) and is considered effective for practical applications (Purcell and Welter, 1990).

In migrant species, migratory success depends both on the quality of dispersal mechanisms and colonizing ability. Selection for successful colonizing ability may alter such demographic features as fecundity and generation time to maximise population growth under colonising conditions. Such changes in population determinants usually result from shifts in developmental aspects of migrant life histories via physiological or behavioural adaptations (Rawlins and Lederhouse, 1981). Thus, the major influence that temperature has on development rates of ectotherms makes it particularly important in the life histories of migrant species.

In *Spodoptera exempta*, the moth lays its eggs in masses of one or more layers on leaves of larval food plants or on other substrates. The number of eggs per mass laid by a female ranges from 10 to 400, but as many as 800 has been reported. The female usually lays more than one egg mass (Brown, 1962; Hattingh, 1941; Whellan, 1960). The egg mass is covered with black (brown, according to Whellan, 1960) hairs ('down') from the abdomen of the female. Hattingh (1941) describes the egg as oblate-spheroidal, pale yellow (Whellan- cream) and shining when first laid. The colour changes with age through brown to black just before hatching. The incubation period varies from two to five days (Hattingh 1941; Faure, 1943; Whellan 1960).

Newly-hatched larvae remain in clusters for the

first few hours. They then disperse by hanging on fine silk threads, a habit which ensures that most of the young larvae land on low growing plants which, in most cases, will be grasses (Whellan, 1960). The larval stage lasts 14-32 days (Brown, 1962) and passes through six instars. Faure (1943) recorded 10-13 days for the larval period. The larvae show a density-dependent phase polyphenism (see Chapter 1).

The mature caterpillar burrows into the soil to pupate. It is enclosed in a delicate cocoon of soil particles held together by silk. The pupal period lasts 7-21 days (Hattingh, 1941; Faure, 1943).

The figures given for the duration of the stages of *S. exempta* life cycle differ from one author to another. Brown (1962) points out that this variation appears mainly to be due to differences in the temperatures under which observations were made. Hattingh (1941) reported that temperatures between 75°F and 90°F (23.8 and 32.2°C) were 'extremely favourable' for the development of *S. exempta* larvae. At temperatures below 75°F development was 'extremely slow'. No larvae could be reared to pupation at temperatures below 60°F (15.5°C).

In his studies on the effect of food plants on the development of larvae, Yarro (1982) observed that the development of *S. exempta* was faster during the months of higher ambient temperature than the months of lower temperature. For a group of larvae reared on maize in June, 1978 (mean temperature, about 16°C) the duration of the larval period was about 85 days, while a period of about 21 days was recorded for larvae reared in January (mean temperature, about 20°C). There were differences in the development periods recorded by Hattingh (1941) and Yarro (1982) which the

latter attributed to the fact that his studies were done under fluctuating temperatures while Hattingh used constant temperatures.

Persson (1981) reported a negative correlation between developmental time and the temperature at three geographical locations in Kenya, a rise in mean monthly temperature of 1°C reducing the developmental period by 3.9 to 5.7 days. Khasimuddin (1981) studied development of *solitaria* and *gregaria* larvae at one cycling (22:10°C) and one constant (25°C) temperature regime. He found that the *solitaria* larvae took longer than *gregaria* to develop at both temperature regimes.

5.1.2 Effect of surface colour on body temperature

Radiative heat gain and convective heat loss are the major avenues of heat exchange for most insects. Evaporative loss is low, and heat exchange by conduction is negligible because of the small surface contact between an insect and solid substrates, especially in adult insects. Convective heat exchange depends on body size and wind velocity (at high speeds) (Casey, 1981).

In sunshine, temperature excess (excess of body temperature over ambient temperature in ectotherms) varies directly with the intensity of radiation to which the animal is exposed. With the variety of wavelengths making up the spectral composition of sunlight, the magnitude of radiation absorbed is affected by a number of factors, including the capacity of the cuticle to absorb energy, the presence or absence of pubescence and surface colouration (Casey, 1981).

The surface colouration of insects may have

important consequences for their thermal balance, as different surface colours could potentially result in large differences in the quantity of radiant energy absorbed. However, the results of studies on the effect of colouration in insects are contradictory. Strelnikov (1936) reported that the body temperature of 'black-brown' locusts were up to 8°C higher than green coloured ones. This supported the results of an earlier experiment by Hill and Taylor (1933). Digby (1955) found that thoracic temperatures of black locusts were significantly greater than lighter forms.

On the other hand, Pepper and Hastings (1952) found higher but insignificant differences between buff and black morphs of *Melanoplus differentialis* (Thomas). In addition, Stower and Griffiths (1966) were not able to demonstrate a consistent difference in equilibrium body temperature or rate of increase in body temperature in paired comparisons between red-dark and green morphs of *Schistocerca gregaria* at several radiation levels. According to Casey (1981), it is possible that the differences in colour between green and red-dark forms were not great enough to account for different levels of absorption of solar radiation from the visible part of the spectrum.

In certain butterflies, only the wing base on the ventral portion of the wings is melanized. Under the same environmental conditions, dark forms achieve equilibrium temperatures about 15% greater than size-matched light forms. These differences in temperature are correlated with greater measured absorption of radiation by the wing base at wavelengths in the visible region (Watt, 1968). *Colias* species inhabiting higher altitudes (regions of lower mean ambient temperatures) exhibit significantly more melanin in

the wing base. Also, the presence of melanin in the wing base shows a seasonal variation. In summer (at higher ambient temperatures), the degree of melanisation of wing bases in *Colias* species from a given habitat is much lower than in the spring and fall (Watt, 1969).

In the pierid butterfly, *Nathalis iole* Boisduval, adults reared in the laboratory under long-day photoperiods emerge in non-melanized (immaculate) form, while shorter photoperiods result in adults with melanized wing bases. As in *Colias* species, melanized adults of *N. iole* exhibit more rapid heating rates and higher equilibrium temperatures than immaculate forms (Douglas and Grula, 1978). These authors concluded that the melanic forms were better adapted to survive and reproduce in cooler thermal environments because they could absorb available radiant energy and maintain flight temperature. In the field, the melanic forms are more common in the spring and fall whereas the immaculate forms are predominant in the summer. This indicates that certain individuals in a population are able to anticipate seasonal changes through 'an adjustment in melanin synthesis mediated by photoperiod' (Douglas and Grula, 1978).

In *Papilio machaon*, differences in equilibrium body temperature occur in dorsal basking individuals before and after the black scales on the dorsal surface of the wing base are removed. The removal of wing scales reduces heat gain by about 14%. Most dorsal baskers have black wing bases regardless of wing colouration (Wasserthal, 1975). In the Namib desert, there are tenebrionid beetles which have either white or jet black elytra. The latter have significantly higher measured abdominal temperatures

than those having white elytra. Measurements of reflectance indicate that white elytra absorb significantly less radiation than black ones (Edney, 1971). Though these observations appear to indicate that the colour difference relates to the thermal ecology of this species, Edney (1971) considers the vivid contrast between white and black as an indication that the white colour has an aposematic role.

Surface colours may also have a role in thermal balance of caterpillars. Casey (1993) points out that it is significant that many basking caterpillars are darkly coloured, though colouration may not necessarily be a thermoregulatory characteristic. Colouration may be changed in response to changing environmental conditions. For instance, in *Ctenucha virginica* (Arctiidae), the colour of the setae changes markedly on a seasonal basis. During the spring and autumn, larvae are black and yellow, but they are predominantly yellow in the summer. The black and yellow caterpillars achieved significantly higher body temperatures than the yellow ones. Thus colour enhanced the body temperatures during cool periods and reduced the temperature excess during the warmer season (Fields and McNeil, 1988).

In other species, physiological colour changes occur in relation to temperature. The Australian alpine grasshopper, *Kosciuscola tristis* Sjost., changes colour in response to different temperatures. Below 15°C, while in a basking posture, the hoppers are 'dull near-black', while at higher temperatures (above 25°C) the surface colour becomes 'bright greenish-blue'. This response is related to the migration of two types of pigment granules in the

epidermial cells in response to temperature. The colour change is less marked in females than in males (Key and Day, 1954 a and b). Similar changes occur in several species of damselflies, and can be observed in as little as 10 minutes after the ambient temperature has changed (O'Farrell, 1963). In the Odonata, their significance in thermoregulation is doubtful. It appears that darker colours facilitate thoracic heating (O'Farrell, 1963), though in some species, they are not considered an effective means for the adjustment of body temperature (Veron, 1973). An alternative hypothesis suggests that the small temperature effect of having a dark colour increases the ability of the insect to detect the direction of incident sunlight and orient more precisely towards it (Veron, 1974).

Johnson *et al.* (1985) reported a relationship between ambient temperature and the degree of melanisation in crowded (*gregaria*) larvae in the cotton leafworm *Alabama argillacea*. Larvae held at 18°C were darker than those held at 27.5°C. Temperature is also known to influence colour patterns in honey bees (Spivak *et al.*, 1992).

5.1.3 Behavioural thermoregulation in insects

A variety of insects control body temperature by behavioural means only, or in combination with endogenous heat production. Larvae of holometabolous insects do not produce heat in sufficient quantity to elevate their body temperature, so regulation by caterpillars is entirely dependent on external heat sources, and ability to regulate varies with species (Casey, 1981). Larvae of *Danaus plexippus* raised body

temperatures 3 to 8°C above ambient by basking behaviour. Such temperature excesses were achieved by orientation of the body to obtain maximum exposure to the rays of the sun and resulted in a reduction of larval duration by 10 to 50% (Rawlins and Lederhouse, 1981). Larvae of *Colias* species also rely on behaviour to raise their body temperatures to levels that are required for activity, especially for feeding. (Sherman and Watt, 1973).

5.1.4 Objectives of Study

Hattingh's (1941) studies are the most detailed reported on the effect of temperature on development of *Spodoptera exempta* but they did not examine the interaction of phase with temperature, as he used only crowded larvae. Khasimuddin's (1981) investigation on phase and development did not cover a wide enough range of temperatures.

Rose (1979) suggested that the black pigment in the *gregaria* larvae of *S. exempta* may be used in the behavioural modification of body temperature. With their preference for feeding in sunlit positions and their basking behaviour, the pigment would absorb solar energy and thus increase body temperature. Higher body temperatures would in turn raise feeding rates and enhance growth and development (Rose, 1979; Casey, 1993). In addition to Khasimuddin's (1981) report on higher development rates of *gregaria* than *solitaria* under the same temperature regimes, Simmonds and Blaney (1986) also mentioned that the *gregaria* develop faster under the same temperatures and radiation load. No investigation has been done on the role of pigment in causing such differences.

Considering the importance of temperature in migratory species (Rawlins and Lederhouse, 1981; McDonald, 1990) and the importance of phase to migration in *S. exempta* (see Chapter 1) it is necessary to investigate the effect of phase on the thermal ecology of this species. The phase change may make alterations to the temperature-development relationship so as to increase the migratory capacity and colonizing ability of the species.

Experiments were performed to investigate:

i) the response to temperature of larval development in *solitaria* and *gregaria*.

ii) the effect of surface colouration on absorption of radiant energy by the larvae.

iii) the behavioural response of *solitaria* and *gregaria* larvae to radiant energy.

5.2 MATERIALS AND METHODS

5.2.1 Effect of temperature and phase on larval development in *Spodoptera exempta*

Larvae were reared in glass jars in incubators, each set at one of eight constant temperatures: 12.5, 17.5, 22.5, 25.0, 30.0, and 35.0, 37.5 and 40°C. Temperatures were constantly monitored using a data logger (Grant Squirrel) and variation was kept within 1°C of the set temperatures. Thus all larvae were reared throughout the duration of the larval period, at one of these temperatures. The photoperiodic regime was 12L:12D in all incubators. *Solitaria* larvae were reared at a density of one per 120 ml jar and *gregaria* at 8 per 500 ml jar, with filter paper at the base and tops of jars as described in Chapter Two. Larvae were provided daily with fresh leaves of maize.

This experiment was carried out in three runs (for logistical reasons) and the results pooled. Fifty *solitaria* were reared at 17.5, 25, 30 and 35°C, 15 were reared at 12.5 and 37.5°C and 20 each at 22.5 and 40°C. For the *gregaria*, 24 were reared at 12.5, 22.5, 37.5 and 40°C; 64 each at 17.5 and 35°C, and 56 at 25 and 30°C. For each run of the experiment, larvae from the same batch of eggs were used for all temperatures. Third generation *solitaria* were used in all runs.

In another experiment, the effect of temperature on the pigmentation of *gregaria* larvae was also studied. Four jars (32) larvae per incubator were set up as described, at 22.5°C and 35°C soon after hatching, and the colour of larvae in the sixth instar recorded.

5.2.2 Effect of surface colour on Larval Body Temperature

The objective of this experiment was to measure the effect of the absorption of radiant energy by larvae of *S. exempta* on their body temperature, and how absorption is influenced by colour differences between *solitaria* and *gregaria*. The study involved measurement of small temperatures of the order of 0.1°C. It was therefore necessary to use a chamber that was free from draughts, as preliminary work had shown that even small air movements could cause the monitored temperature signal to fluctuate considerably.

For this reason, a chamber was constructed with three of its vertical sides, and the base, made of plywood. The thickness of the plywood was about 1.0 cm on the sides and 0.5 cm at the base of the chamber. The base of the chamber was lined with a 2.5 cm thick slab of polystyrene bonded to the wood. The top of the chamber was made of a thin (0.2 cm) glass sheet to minimise the amount of incident radiant energy that it would absorb. The front panel was also made of glass, and could be raised (by sliding through a groove in the wooden sides) to introduce or remove larvae. The internal dimensions of the box were 25.5 x 22.2 x 20.3 cm.

The light or energy source used to determine the rates of absorption by larvae was a 100 W tungsten-filament light bulb which was placed approximately 8 cm above the chamber, about 28 cm above the larvae. By experimentation, this had been found to be the most suitable height for producing a relatively fast increase in temperature of the animals before the air in the chamber attained the same temperature. Thus the

larvae would heat up primarily by radiation rather than the air (convection). An incandescent source was used because it is rich in infra red radiation which was assumed to affect the body temperature more than shorter wavelengths. UV light could cause more changes at the molecular level without much effect on the body temperature.

Three extremely fine copper-constantan thermocouples were made and their junctions inserted and bonded, with superglue, into 12 x 0.4 mm hypodermic syringe needles. The three needles were mounted at the base of the chamber so that they projected about 2.5 mm (about one half the mean thickness of a sixth instar larva) above the polystyrene floor. There was a gap of about 1.5 cm between needles. To the centre thermocouple was attached a small (1.8 x 1.1 cm) rectangular piece of aluminium sheet whose surface was painted matt black. This centre thermocouple was used as a reference (the standard 'blackbody') against which the two others were compared. The three thermocouples also had their respective co-junctions maintained at 0°C in a thermos flask containing a mixture of crushed ice and distilled water. The free end of each thermocouple was connected to a channel on a Rikadenki multichannel chart recorder. Pens of different colour were attached to three channels on the recorder, black for the reference and red and blue for the other two. The sensitivity obtained for the recorder was a displacement of 7 mm for 1°C change in temperature. The reference thermocouple was also monitored externally by means of a hand-held digital thermometer (Kane-May), with an accuracy of 0.1°C. Its readings were used as a cross-reference to those on the chart

recorder.

Before any records were taken, the accuracy and linearity of the thermocouples were determined. The chart recorder and digital thermometer were switched on and allowed some time to stabilise. Then the channels were adjusted to a common starting position and the temperature indicated on the digital meter noted. The lamp was turned on and the changes in temperature closely monitored for about one hour. The temperature indicated on the digital meter was marked on the chart at regular intervals during the run. A linear regression of displacement on temperature was then obtained for each of the thermocouples from the temperatures indicated and the corresponding deflections on the chart. The coefficients of regression were extremely high (r = 0.99965, 0.99968 and 0.99991 for the blue, red and reference channels respectively) meaning that linear temperature measurements could be expected. The slope for each channel was also obtained so that temperatures could be obtained by interpolation.

A set of experimental runs were then made for larvae belonging to one of five main colour bands : green, green-brown, and brown *solitaria* and green and black *gregaria* (see Fig 5.5 and 5.6). Allocation to these colour bands was subjective. Two larvae of about the same size were exposed to methyl acetate in a jar to anaesthesize them. This was necessary to prevent movements of the larvae during measurements. One larva was then stuck on each of the needles on either side of the blackbody, so that the dorsum was perpendicular to the rays from the lamp. Changes in temperature of the blackbody and the larvae were then

recorded on the chart. During these runs, the temperature indicated on the digital meter was marked on the chart periodically to check that the slope of the chart recorder's sensitivity remained constant.

There were slight differences between the initial slopes of the different channels, due to differences in the tolerances of the thermocouples. Therefore, it was necessary to normalise the traces from the chart recorder. These curves were first digitised with a digitising tablet (GTCO inc.) and cursor and the data saved as files on an IBM PC computer. Software was written using Turbo Pascal version 6 to normalise data from the three channels and these normalised data were output to a (HP 7470A) plotter. The 'y' displacement of each curve then indicated the correct temperature attained by each larva against time. The reference thermocouple was the indicator for the rate of heating during each experimental run.

The data for all larvae in the same colour category were then pooled and the rates of increase in temperature due to radiation compared.

5.2.3 Behaviour of *solitaria* and *gregaria* larvae under radiant energy

This experiment was carried out in a 'radiation room' which was fitted with a solar simulator made up of two 150 W metal halide lamps and four 250 W infra red (IR) lamps mounted about 0.9m above a table. The lamps enclosed an area of about 1 m^2 with one IR lamp at each corner of the rectangle. One metal halide lamp was mounted between each pair of IR lamps on opposite sides of the rectangle. The mean radiant load from this simulator measured as irradiance (reflected flux)

with the IRIS MK IV instrument, was 544.1 mW m^{-2}.

Cardboard boxes measuring 30 x 22 x 23.5 cm filled with loosely packed hay to about 9 cm from the top, were placed under the lamps. These boxes were stood on trays with pots containing water. Entire maize leaves were then passed through slits in the base of each box through the hay pack so that the upper ends of the leaves showed above the hay and their lower ends dipped in the pots of water. The object of this arrangement was to simulate a mat of herbage in the field.

Samples of sixth-instar *solitaria* and *gregaria* larvae were then placed in the cardboard boxes, after being kept for about an hour in the 'radiation room' to allow them to adjust to the conditions in that room. These larvae were reared under the same conditions as the main culture (see Chapter 2) till they reached the sixth instar. Holes were made (one for each *solitaria* and two for the *gregaria*) on one side of each box, 3-4 cm above the base. The larvae were placed in 55 ml plastic containers which were inserted into the holes to allow the larvae to enter the boxes. The holes were made directly opposite a maize leaf, so that the larvae encountered a leaf as soon as they entered the box. The lamps were turned on one hour after the larvae had been placed in the boxes. This procedure was repeated, and for each trial 10 *solitaria* (one per box) and ten *gregaria* (all in one box) were used.

Observations were made at 30-minute intervals on the proportion of larvae which were exposed to the lamps or were above the hay both before and after the lamps had been switched on. Exposure was scored as full or partial, the latter referring to larvae on the

abaxial surface of the leaf or in any way shaded from the full impact of energy from the lamps.

Body temperatures of a sample of eight *solitaria* and six *gregaria* larvae were also measured. This was done by pressing a thermocouple probe into an intersegmental groove on the abdomen of the larva. The pressure takes the thermocouple junction to the core of the body so that approximate internal temperatures could be obtained without actually penetrating the cuticle (Rawlins and Lederhouse. 1981). *Solitaria* larvae tend to be larger than *gregaria*, but attempts were made to use larvae that were as closely size-matched as possible, as the rate of increase in body temperature is related to size (Sherman and Watt, 1973; Kevan *et al*., 1982).

5.3 RESULTS

5.3.1 Effect of Temperature and phase on larval development

Table 5.1 gives the mean durations of the larval period at the seven temperatures for which data were collected. No larvae survived till pupation at 12.5°C. At 40°C, some *solitaria* pupated, but no moths emerged from these pupae. *Gregaria* larvae failed to pupate at 40°C. For both phases, the rate of development increased (and larval period decreased) with temperature from 17.5°C to 30°C (Table 5.1; Figs. 5.1 and 5.2). The development rate decreased with increase in temperature above 30°C, but the fall between 30 and 35°C was not significant for the *gregaria* larvae. Percentage development per day is the development rate (inverse of the development period) multiplied by 100 (Arnold, 1959).

The rate of development was higher for the *gregaria* from 17.5°C to 25°C. Between 25°C and 30°C, there was a switch over, resulting in the *solitaria* having higher rates of development than the *gregaria* at 30°C. Above this temperature, there was no difference in rate of development between phases (Table 5.2).

Figure 5.3 shows the predicted rates of development of *solitaria* and *gregaria* larvae at 30°C and below. The lines in this figure were obtained by regression of percentage development per day (y) on temperature (x). From this relationship, it is possible to calculate a theoretical threshold for development at which the development rate is zero, and

hence the 'physiological time' required for larval development (Arnold, 1959; Roberts and Mahr, 1986; Begon et al, 1990; Miller and Maddox, 1991; Barker and Enz, 1993). The correlation coefficients are significant for both lines in Fig. 5.3 (r= 0.9565 for *solitaria* and r= 0.9235 for *gregaria*) The predicted rates of larval development are based on the temperature-development relationship from 17.5°C to 30°C, because above 30°C, the development rate falls (Fig 5.2).

Between 17.5°C and 30°C, the equation for regression of percent development per day (y) on temperature (x) is:

$$y = -5.36 + 0.423\,x$$, for the *solitaria*

and $$y = -3.89 + 0.369\,x$$, for the *gregaria* larvae.

These equations give development thresholds 12.7° C (5.36/0.423) for the *solitaria* and 10.5°C (3.89/0.369) for the *gregaria*. Hence, the number of day-degrees required for larval development (the product of the predicted development period and the difference between the ambient and 'threshold' temperatures) is 235.6 ± 0.23 (mean ± s.e) for the *solitaria* and 272.0 ± 0.22 for the *gregaria*. Using the mean development periods obtained in this experiment, the required number of degree days above the threshold temperature is 239.5 ± 13.3 (mean ± s.e) for *solitaria* and 283.6 ± 24.5 for the *gregaria*. The predicted value is higher for the *gregaria* (P = 0.000), but there is no significant difference between the observed values for the two phases (F = 2.50, P = 0.165) or between calculated and observed values for either phase (*solitaria*, F = 0.08, P = 0.781; *gregaria*, F = 0.22, P = 0.653).

Table 5.1 Variation with temperature of duration of larval stage in *solitaria* and *gregaria* larvae of *S. exempta*. 'P' indicates the level of significance of the difference in larval period between phases at each temperature (oneway ANOVA):* - 0.05 ≥ P ⟩ 0.01, ** - 0.01 ≥ P ⟩0.001, *** - P ≤ 0.001, ns - non-significant.

	Solitaria		*Gregaria*		
Temp (°C)	n	mean ± se	n	mean ± se	P
17.5	18	56.4 ± 1.45	16	50.1 ± 1.14	**
22.5	13	21.0 ± 0.39	14	19.9 ± 0.38	*
25.0	35	19.6 ± 0.42	25	17.8 ± 0.27	***
30.0	39	13.9 ± 0.18	27	14.8 ± 0.33	*
35.0	28	15.1 ± 0.26	27	15.2 ± 0.32	ns
37.5	10	15.7 ± 0.44	10	16.6 ± 0.62	ns
40.0	7	15.9 ± 0.43	-	-	-

Table 5.2 Mean percentage development per day of *solitaria* and *gregaria* larvae of *S. exempta* at constant temperatures

	Solitaria		*Gregaria*	
Temp (°C)	n	mean ± se	n	mean ± se
17.5	18	1.8 ± 0.04	16	2.0 ± 0.04
22.5	13	4.8 ± 0.08	14	5.1 ± 0.10
25.0	35	5.2 ± 0.10	25	5.7 ± 0.09
30.0	39	7.3 ± 0.10	27	6.9 ± 0.15
35.0	28	6.7 ± 0.11	27	6.7 ± 0.14
37.5	10	6.4 ± 0.19	10	6.1 ± 0.23
40.0	7	6.3 ± 0.17	-	-

Figure 5.1 Effect of ambient temperature and phase on total larval period in *S. exempta*

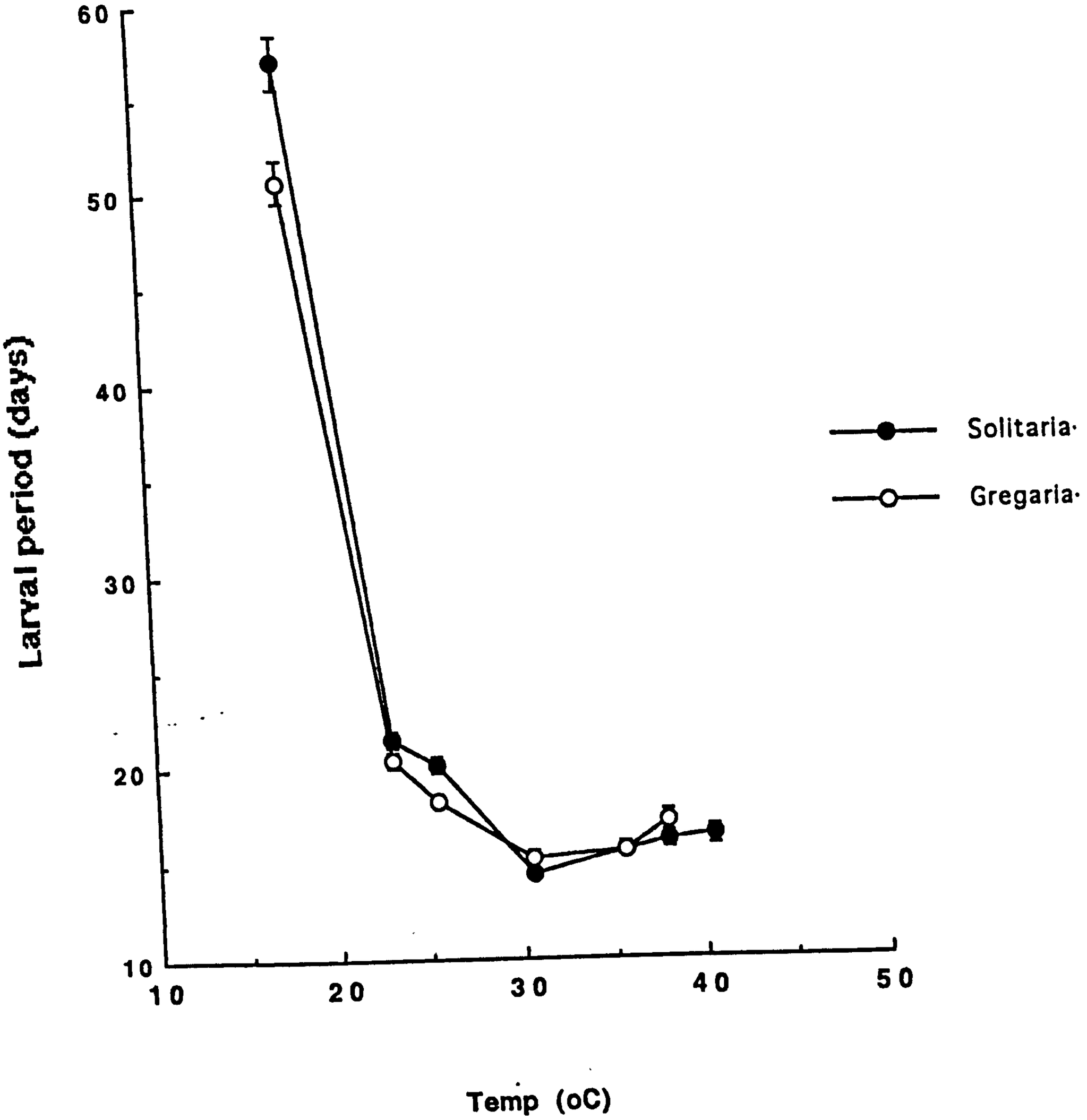


Figure 5.2 Effect of ambient temperature and phase on larval development rate in *S. exempta*

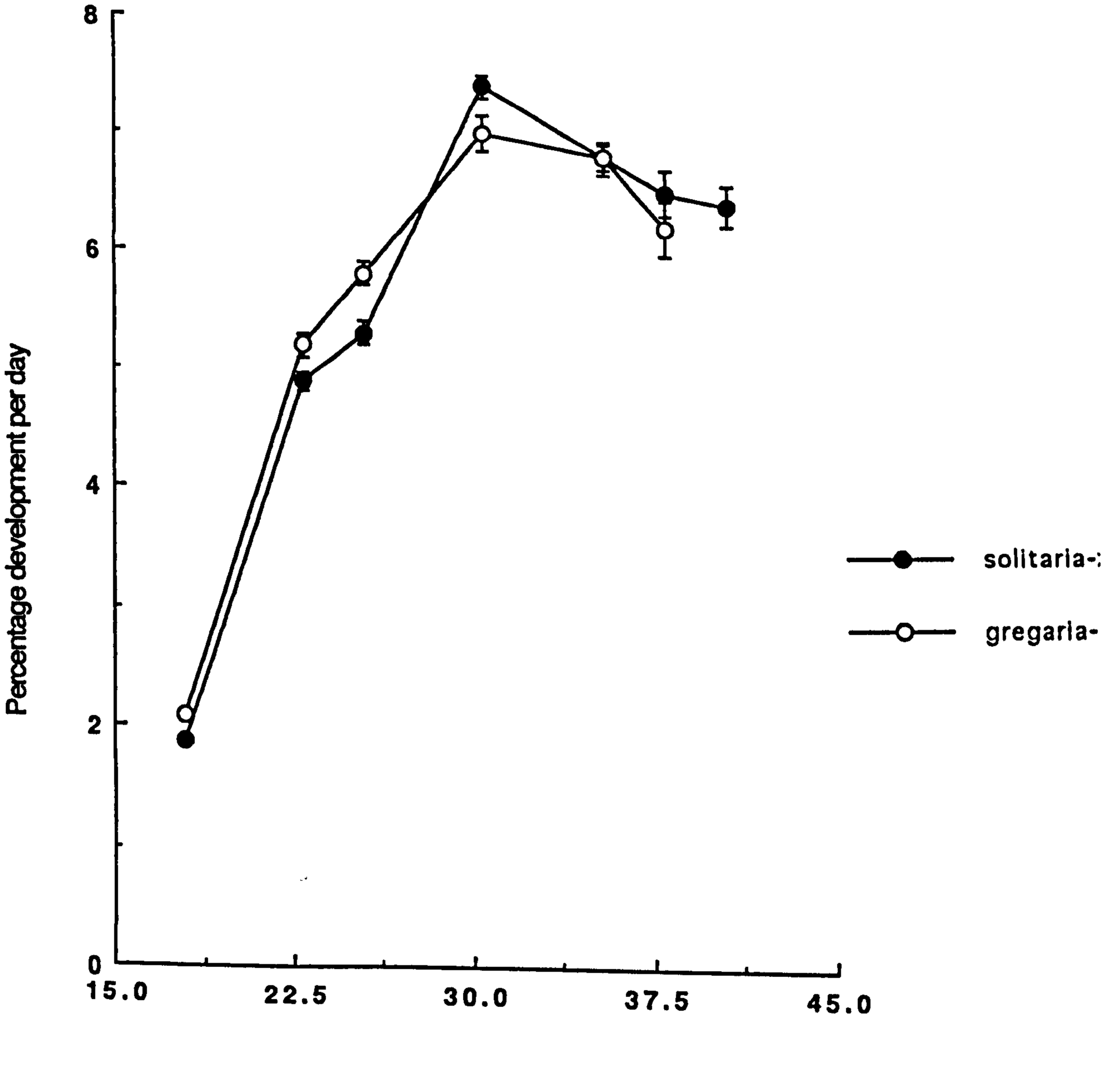
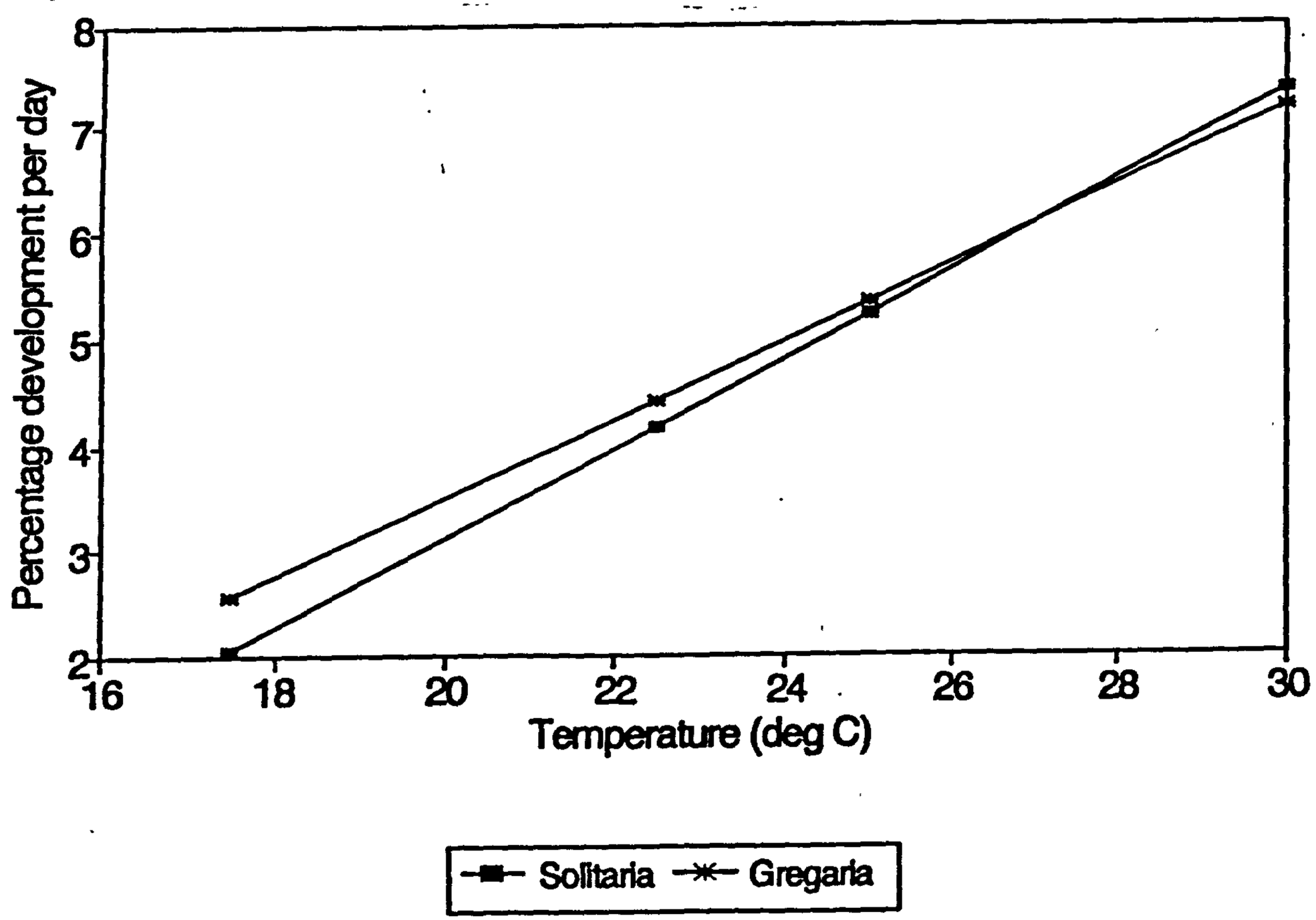


Figure 5.3 Predicted larval development at constant temperatures in *S. exempta*



In the experiment on temperature and pigmentation, all the 21 *gregaria* that survived till the sixth instar at 22.5°C were black. Of the 17 survivors at 35°C, 14 (82.3%) were pale green (see Figure 5.6) and the rest had a darker shade of green. No black larvae were obtained at 35°C. Generally, *gregaria* larvae were darker at 17.5°C and 22.5°C than at 25°C and 30°C. At 35°C and above, the *gregaria* larvae lost the black pigment.

5.3.2 Effect of surface colour on Larval Body Temperature

Tables 5.3 and 5.4 and Figure 5.4 give a summary of mean increases above initial temperature, attained by larvae in the five colour categories.

Table 5.3 Temporal change in temperature of *solitaria* larvae of different colour exposed to energy from an incandescent lamp. Mean increase in temperature is the difference between initial body temperature and the temperature at the stated time.

	LARVAL COLOUR/ MEAN INCREASE IN BODY TEMPERATURE (°C) ± SE		
TIME (MIN)	GREEN	BROWN	GREEN-BROWN
1	1.7 ± 0.07	1.7 ± 0.04	1.6 ± 0.04
2	3.0 ± 0.13	3.1 ± 0.06	2.9 ± 0.07
4	5.2 ± 0.12	5.2 ± 0.07	5.2 ± 0.11
6	6.7 ± 0.07	6.8 ± 0.08	6.8 ± 0.14
8	8.0 ± 0.06	8.1 ± 0.10	8.1 ± 0.12
10	9.0 ± 0.11	9.1 ± 0.11	9.0 ± 0.11
12	9.7 ± 0.08	9.9 ± 0.11	9.8 ± 0.13
14	10.5 ± 0.13	10.5 ± 0.12	10.6 ± 0.12
16	11.0 ± 0.18	11.1 ± 0.12	11.2 ± 0.11
18	12.0 ± 0.40	11.6 ± 0.12	11.7 ± 0.12
20	12.4 ± 0.46	11.9 ± 0.13	12.1 ± 0.15
N =	6	10	10

Figure 5.4 Effect of surface colour on the body temperature of *S. exempta* larvae

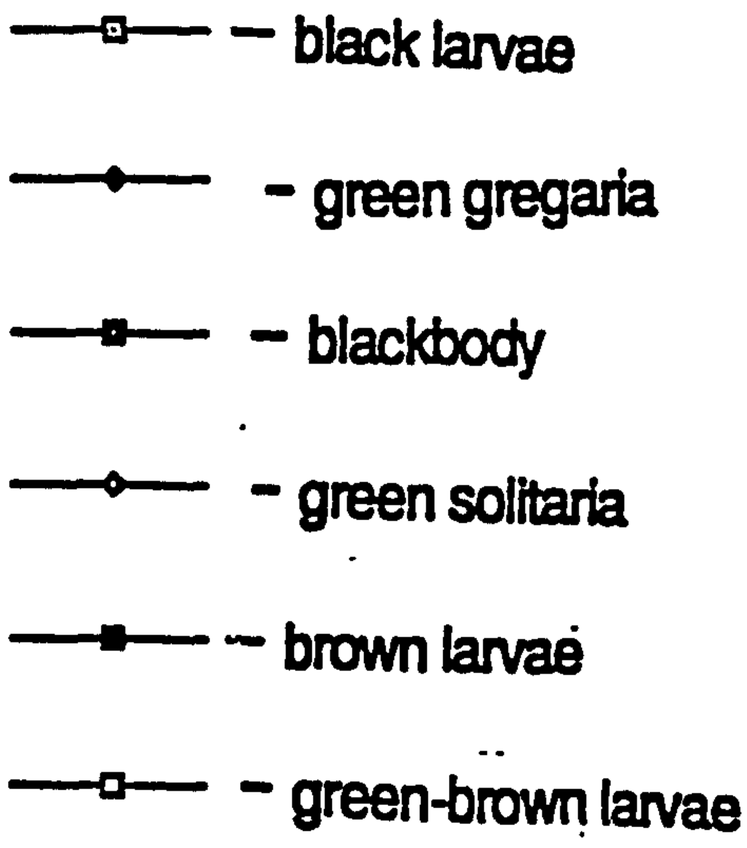


Figure 5.4

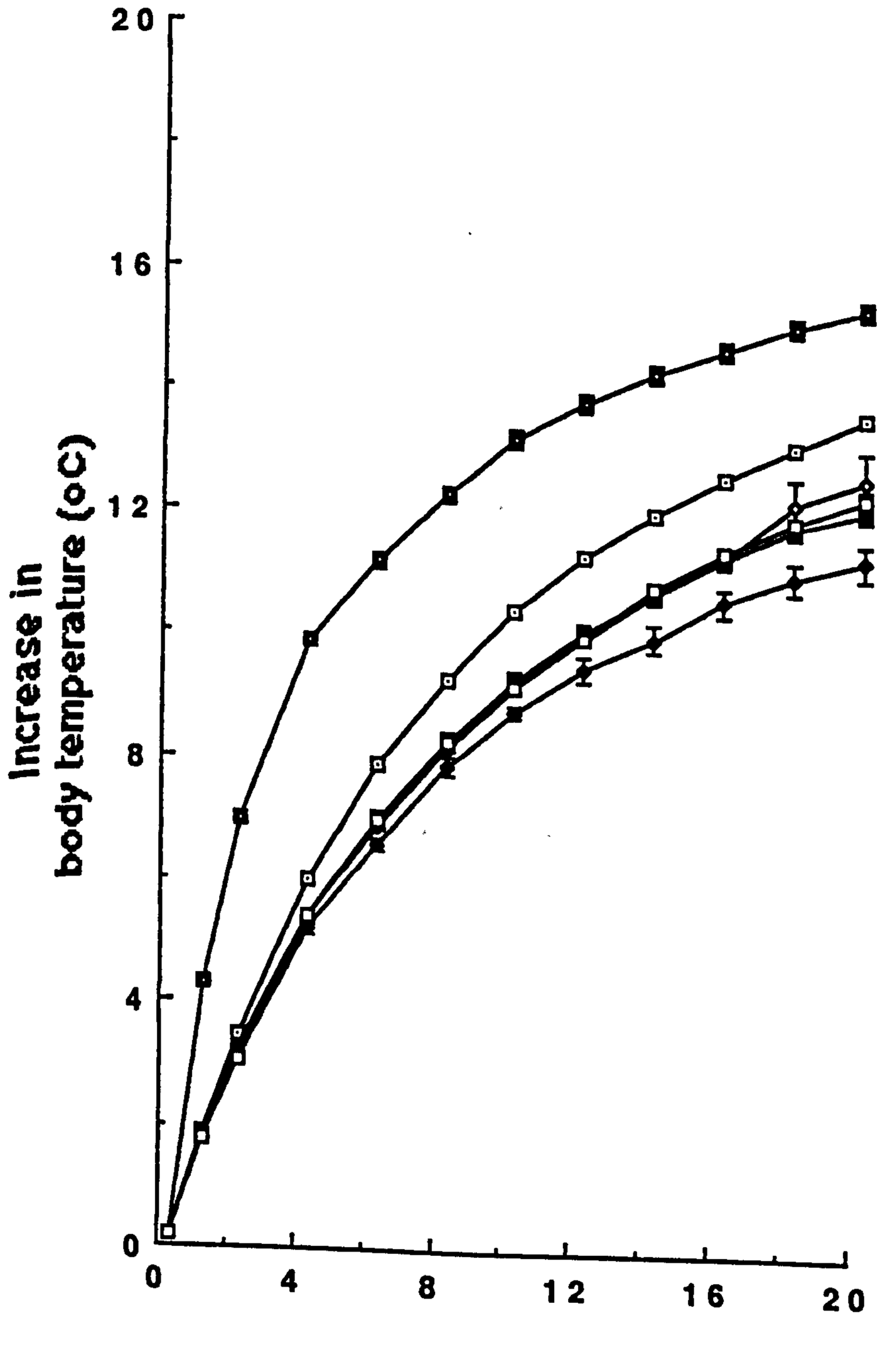

20
16
12
8
4
0
0
4
8
12
16
20
Increase in body temperature (oC)
Time (min)

Table 5.4 Temporal change in temperature of *gregaria* larvae of different colour and a 'blackbody' exposed to energy from an incandescent lamp. Mean increase in temperature is the difference between initial body temperature and the temperature at the stated time.

	LARVAL COLOUR/ MEAN INCREASE IN BODY TEMPERATURE (°C) ± SE		
TIME(min)	BLACK	GREEN	'BLACKBODY'
1	1.7 ± 0.04	1.6 ± 0.03	4.1 ± 0.05
2	3.2 ± 0.07	2.9 ± 0.06	6.8 ± 0.07
4	5.8 ± 0.06	5.0 ± 0.08	9.7 ± 0.10
6	7.7 ± 0.05	6.4 ± 0.09	11.0 ± 0.13
8	9.1 ± 0.06	7.7 ± 0.15	12.1 ± 0.16
10	10.1 ± 0.08	8.6 ± 0.17	13.0 ± 0.13
12	11.1 ± 0.07	9.3 ± 0.20	13.6 ± 0.15
14	11.8 ± 0.09	9.8 ± 0.22	14.1 ± 0.14
16	12.4 ± 0.08	10.4 ± 0.24	14.5 ± 0.16
18	12.9 ± 0.09	10.8 ± 0.25	14.9 ± 0.15
20	13.4 ± 0.10	11.1 ± 0.26	15.2 ± 0.15
N =	13	10	20

Figure 5.5 Photographs showing the range of colours among larvae reared *solitaria* in this study. 'Category' is as indicated in the text (Section 5.3.2) (scale not the same for all photographs).

A: green larva (category 1)

B: green larva with mid-dorsal brown line (category 1)

C: green-brown larva (category 2)

D-F: brown larvae of different shades (category 3)

Figure 5.5

Figure 5.6 Photographs showing the colour contrast between *solitaria* and *gregaria* larvae reared at the same temperature or *gregaria* larvae reared at different constant temperatures (scale not the same for all photographs).

G: *gregaria* larva reared at 35°C (category 4)

H: *gregaria* larva reared at 17.5°C (category 5)

I: *gregaria* larva reared at 37.5°C

J: *solitaria* larva reared at 22.5°C (category 1)

K: *gregaria* larva reared at 22.5°C (category 5)

L: the darkest larva among a batch of *gregaria* reared at 35°C (category 4)

M: *gregaria* larva reared at 22.5°C (category 5)

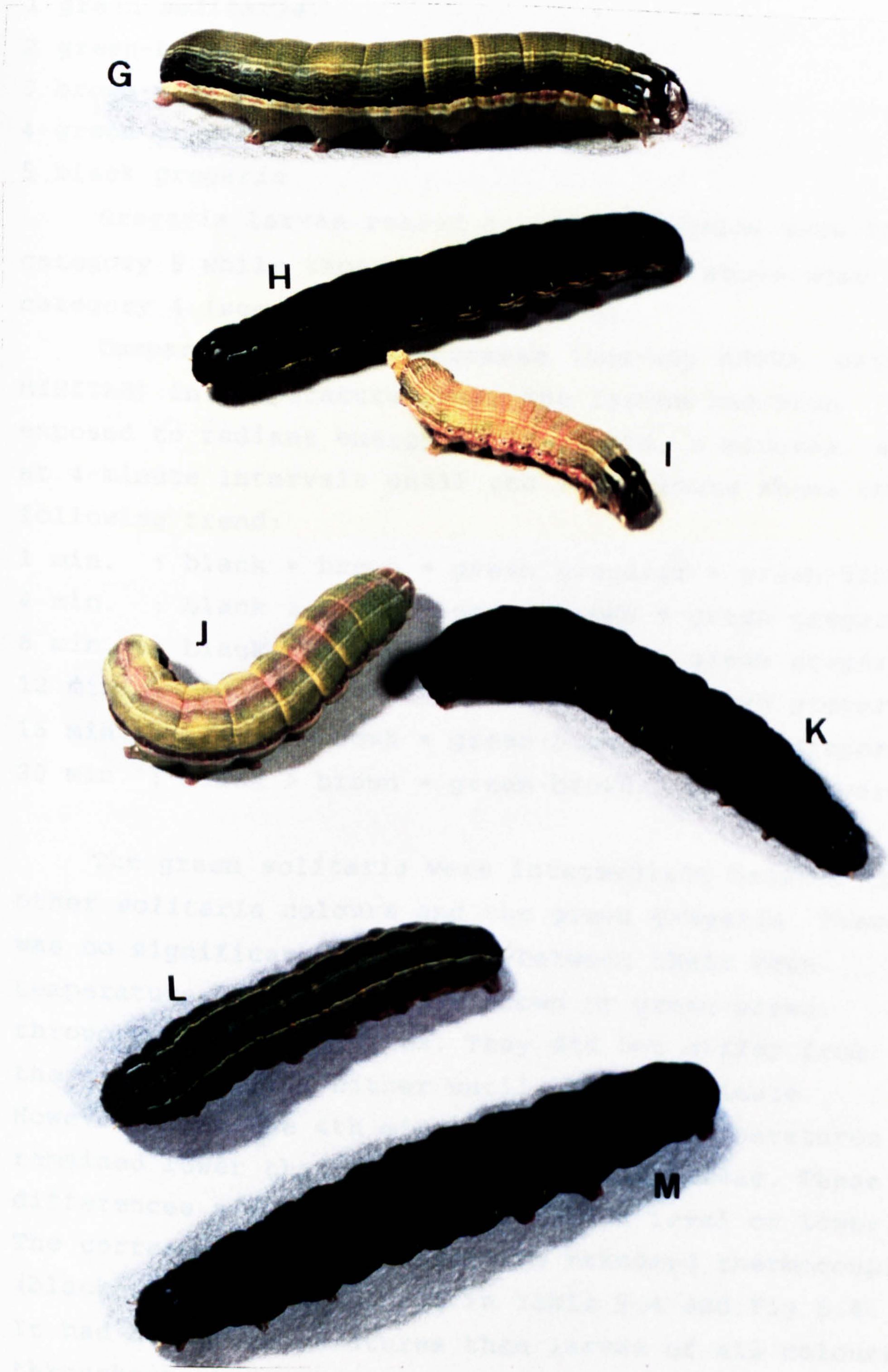


Figure 5.6

The colour categories are:

1 green *solitaria*

2 green-brown *solitaria*

3 brown *solitaria*

4 green *gregaria*

5 black *gregaria*

Gregaria larvae reared at 30°C and below were in category 5 while those reared at 35°C or above were in category 4 (see Figures 5.5 and 5.6).

Comparison of mean increase (one-way ANOVA, using MINITAB) in temperature after the larvae had been exposed to radiant energy for 1 minute, 4 minutes, and at 4-minute intervals until the 20th minute shows the following trend:

1 min. : black = brown = green *gregaria* = green-brown

4 min. : black > brown = green-brown = green *gregaria*

8 min. : black > brown = green-brown = green *gregaria*

12 min. : black > brown = green-brown > green *gregaria*

16 min. : black > brown = green-brown > green *gregaria*

20 min. : black > brown = green-brown > green *gregaria*

The green *solitaria* were intermediate between the other *solitaria* colours and the green *gregaria*. There was no significant difference between their mean temperature and that of the brown or green-brown throughout the 20 minutes. They did not differ from the green *gregaria* either until the 20th minute. However, from the 4th minute their body temperatures remained lower than those of the black larvae. These differences are significant at the 5% level or lower. The corresponding values for the standard thermocouple (blackbody) are also shown in Table 5.4 and Fig 5.4. It had higher temperatures than larvae of all colours throughout the 20-minute test period.

5.3.3 Behaviour of *solitaria* and *gregaria* larvae under radiant energy

Table 5.5 and Figs 5.7 and 5.8 summarise the responses of twenty larvae in the two phases when exposed to energy from the solar simulator over two days. Records were taken just after the last larvae had been put into their boxes. The first hour was without light from the lamps.

Table 5.5 Proportion of *solitaria* (Sol) and *gregaria* (Grg) larvae exposed to radiant energy at various time intervals and their level of exposure (full or partial). n = 20.

	LARVAE EXPOSED (PERCENT TOTAL)				
TIME (h)	0.0-1.0	1.5-2.5	3.0-4.0	4.5-5.5	6.0-7.5
DAY 1					
Sol Full	15.0	17.5	5.0	12.5	10.0
Sol Part.	2.5	22.5	25.0	45.0	35.0
Grg Full	30.0	47.5	20.0	65.0	45.0
Grg Part.	0.0	22.5	42.5	27.5	20.0
DAY 2					
Sol Full	30.0	60.0	50.0	57.5	40.0
Sol Part.	10.0	22.5	45.0	30.0	57.5
Grg Full	15.0	35.0	42.5	52.5	67.5
Grg Part.	17.5	7.5	25.0	0.0	25.0

More *gregaria* than *solitaria* were exposed throughout the first day of observation. The total number of exposed larvae in both phases appeared to follow the same pattern. It rose after the first hour,

Temporal changes in proportion and extent of larval exposure to radiant energy

Figure 5.7

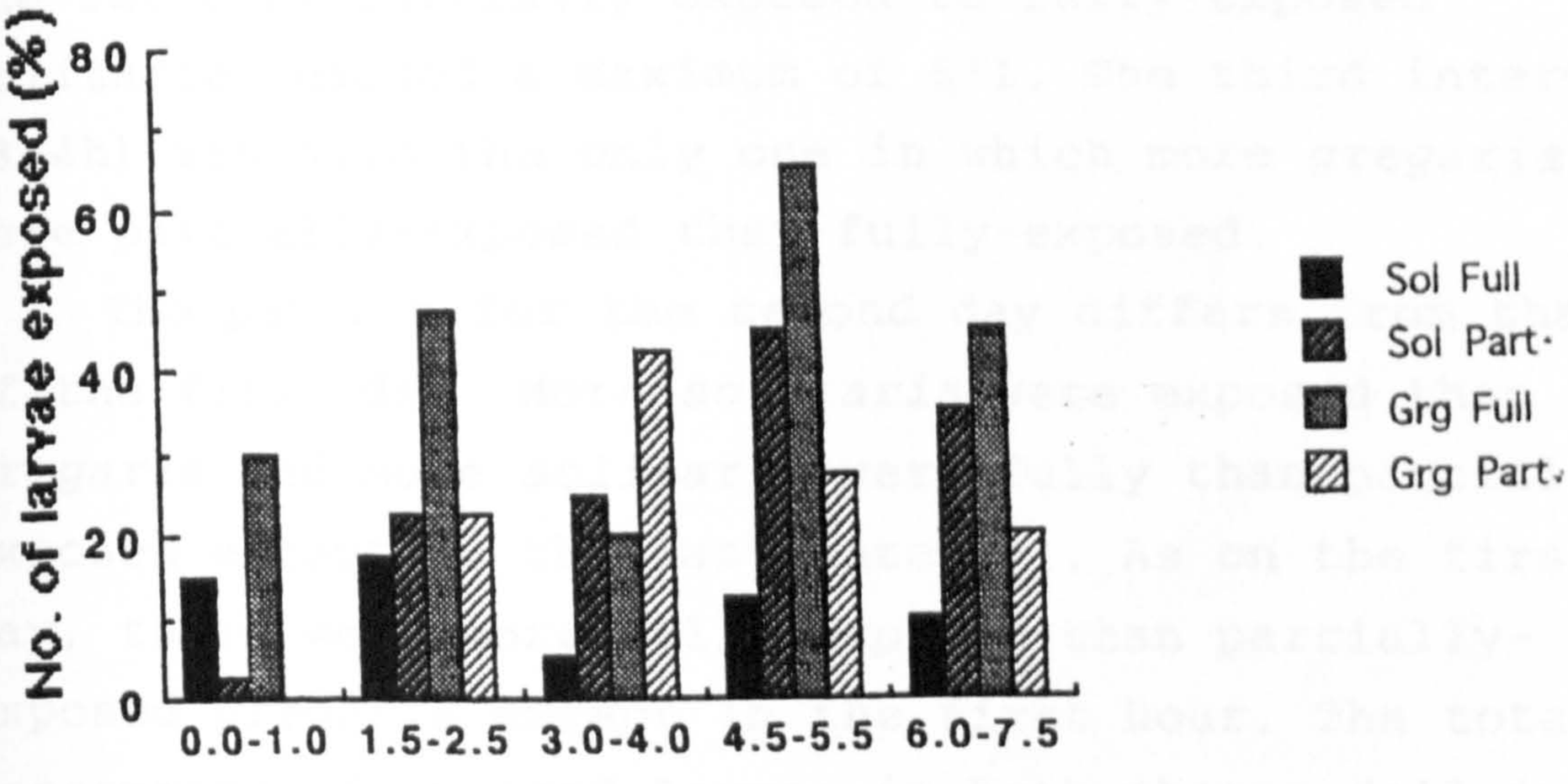
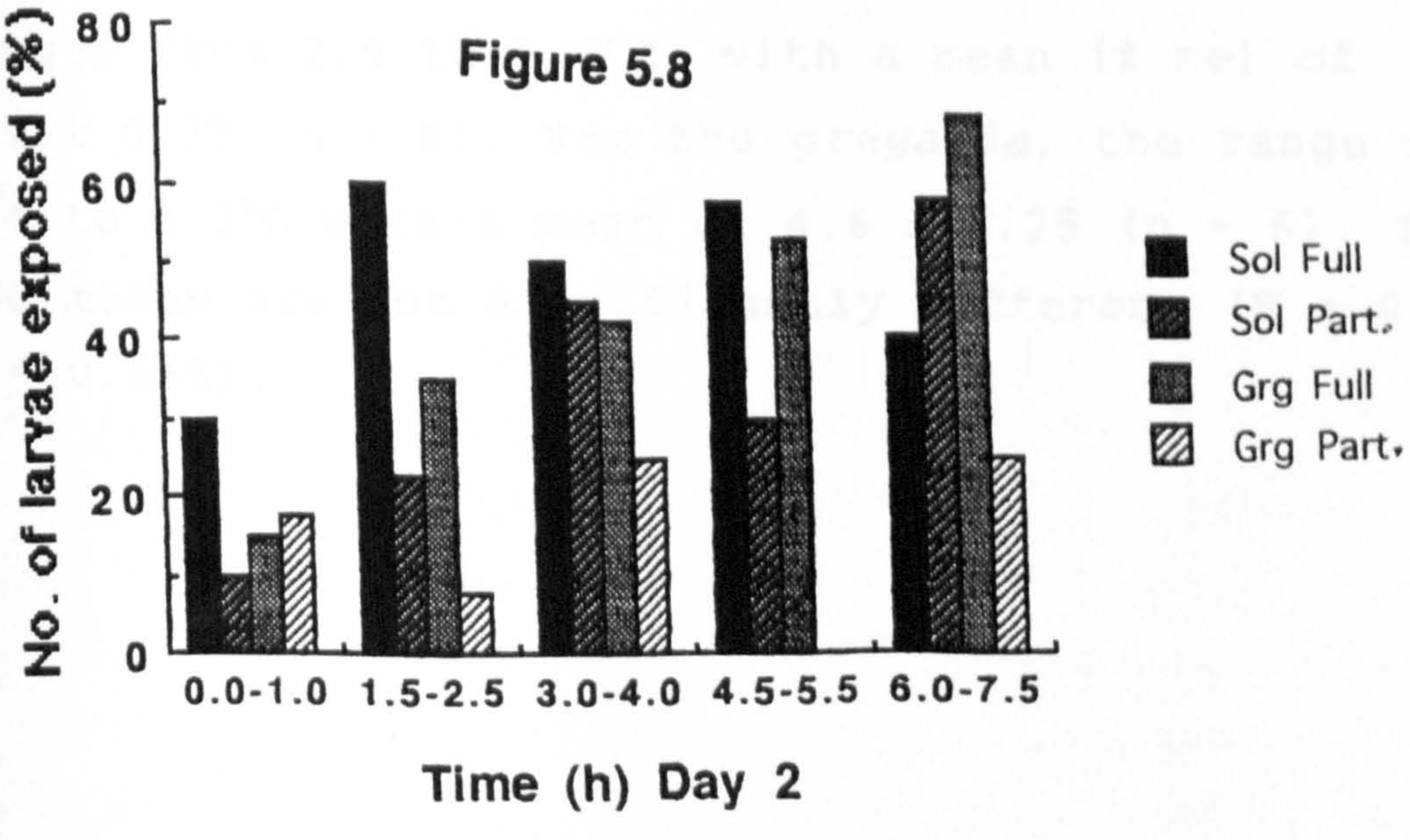


Figure 5.8



when the lamps were turned on. This was followed by a reduction, another increase and finally a fall in the last interval (6.0 - 7.5 h). The difference between phases seemed to be in the level of exposure. After the first hour, the proportion of partially-exposed *solitaria* was always higher than the percentage which was fully exposed. Between the third and fourth hours, the ratio of partially-exposed to fully-exposed *solitaria* reached a maximum of 5:1. The third interval (3-4h) was also the only one in which more *gregaria* were partially-exposed than fully-exposed.

The pattern for the second day differs from that of the first day. More *solitaria* were exposed than *gregaria* and more *solitaria* were fully than partially exposed except in the last interval. As on the first day, there were more fully-exposed than partially-exposed *gregaria* except in the first hour. The total percentage of exposed larvae in both phases fell in the fourth interval (4.5-5.5 h).

The body temperature excesses (difference between body and ambient temperatures) for the *solitaria* ranged from 2.9 to 5.6°C, with a mean (± se) of 4.4 ± 0.37 (n = 8). For the *gregaria*, the range was 3.4 to 5.1°C with a mean of 4.6 ± 0.25 (n = 6). The two means are not significantly different ($F = 0.17$, $P = 0.685$).

5.4 DISCUSSION

The *gregaria* larvae developed faster than the *solitaria* at lower temperatures: 17.5 to 25°C. At 30°C, the *solitaria* have a higher rate of development, but this falls off relatively quickly to become equal with the rate of development of the *gregaria* at 35°C.

The number of day degrees (239.5 for *solitaria* and 283.6 for *gregaria*) required for development is not significantly different for the two phases. This means that they require the same duration of physiological time above threshold, which the *gregaria* accumulate in a shorter period of time at temperatures below 30°C. The implication of this is that the *gregaria* are better adapted for development at lower temperatures. At least two findings of this experiment support this inference. First, the theoretical lower threshold temperature is lower (10.5°C) for the *gregaria* than for the *solitaria* (12.5°C). Secondly, the *gregaria* lose their black pigment at higher temperatures (that is above 35°C) and look darker at lower temperatures (17.5 and 22.5°C) than at 25 and 30°C.

From the above, it should be expected that whether or not a difference is found between development times of *solitaria* and *gregaria* in an experiment will depend on the rearing temperature. Gunn and Gatehouse (1987) reported that there were no significant differences in development times in their cultures reared at 28°C and drew a comparison with Simmonds and Blaney's (1986) finding that *gregaria* insects developed faster than *solitaria* larvae in experiments carried out at 25°C. From Figure 5.2 and Table 5.2, it is clear that the *gregaria* have a higher

development rate at 25°C, while 28°C falls in the region where the development rate of the *solitaria* catches up with that of the *gregaria*. Also in agreement with this view is Khasimuddin's (1981) finding that *solitaria* developed more slowly at a cycling temperature regime of 22:10°C.

As has been shown in *Colias* species (Watt, 1968), monarch butterflies, *Danaus plexippus* (Rawlins and Lederhouse, 1981), *Nathalis iole* (Douglas and Grula, 1978), larvae of *Ctenucha virginica* (Fields and McNeil, 1988), alpine grasshoppers (Key and Day, 1954 a, b) and some Odonata (O'Farrell, 1963; Veron, 1973; 1974), dark colouration is an adaptation for survival and development in environments with low ambient temperatures. It may seem doubtful that a tropical species would require such an adaptation. However, outbreaks of armyworm are known to occur in east Africa from November to about June of the following year. Yarro (1982) gives temperatures of 16°C and 20°C for June and January, respectively, at Chiromo in Kenya. Therefore, the temperatures in at least parts of the range of the African armyworm (during the outbreak season) are lower than 30°C, thus falling in the range for which having the black pigmentation is still an advantage. It is also pertinent that larval outbreaks occur in the rainy season, when it is likely to have clouds that cut off direct insolation during the day. Again it would be an advantage to have a pigment that would boost the rate of absorption of radiant energy even under such conditions.

Rawlins and Lederhouse (1981) explain why the temperature-development relationship is particularly important to migrant species, based on their work on *Danaus plexippus*. Larval behaviour that raises larval

body temperatures above ambient, would reduce larval duration and generation time. Reduction of larval duration lowers mortality by shortening the total time caterpillars are exposed to parasitoids, predators or disease. By reducing generation time, rate of population growth is increased, so that populations can expand rapidly into regions and in seasons where larval host plants are abundant (Rawlins and Lederhouse, 1981). Similarly, the *gregaria* larvae of *S. exempta* seem to be adapted to reduce the effect, on development, of low ambient temperatures so that generation time is not unduly prolonged. Thus development will be as rapid as possible under all environmental conditions the larvae are likely to encounter.

The results of the experiments on surface colour and body temperature (Tables 5.3, 5.4; Fig. 5.5) suggest that the black pigment of the *gregaria* larvae contributes to their higher development rate, as black larvae attained higher temperatures than larvae of other colours. When *gregaria* larvae lost the black pigment during development at temperatures in the upper range (35°C and above), they no longer attained higher temperatures under radiant energy (Fig 5.5) nor did they develop more quickly than *solitaria* in the absence of radiant energy (Fig 5.2).

According to Casey (1993), the occurrence of phase polymorphism in *S. exempta* involves a change of thermal strategy from thermoconformation to thermoregulation. He is of the opinion that the different growth rates by the phases at constant temperature indicate an alteration of the thermal sensitivity of important physiological processes, in addition to a change from a cryptic thermoconforming

strategy to that of a thermoregulator.

Casey's (1993) proposal of a change in thermal strategy agrees with the idea that the effect of the black pigment in the *gregaria* would be enhanced by their preference for feeding in sunlit positions (Rose, 1979). The results of the experiments on behaviour (section 5.3.3) do not clearly indicate any difference between *gregaria* and *solitaria* larvae in their behavioural responses to radiant energy. *Solitaria* larvae did not exhibit any tendency to avoid exposure to radiant energy in the laboratory. However the laboratory set-up does not simulate all the conditions in the field. For instance, the effect of predators which may select for more cryptic behaviour, is not provided for. The spectral characteristics of the lamps used probably did not come close enough to those of natural sunlight.

That the temperature excesses recorded for larvae in this experiment did not seem to be associated with their pigmentation appears to contradict the earlier findings (Tables 5.3, 5.4) on immobilised larvae. The temperature measurements on active larvae (traces on the chart recorder) were not as smooth as those on the immobile larvae, because the former were affected by the movements of the larvae as well as by air movements, no matter how little. Also unlike the immobile larvae in an enclosure, the mobile larvae had not been exposed to equal intensities of radiant energy for equal lengths of time.

The experiments in this chapter have demonstrated that *gregaria* larvae develop faster than *solitaria* but only at temperatures up to 25°C (in the range studied). There is some evidence that this difference in development rates may be enhanced under natural

conditions due to higher rates of absorption of radiant energy by the black pigment in the *gregaria*. At higher temperatures, the development of the black pigment by *gregaria* larvae is reduced. However, the role of behaviour in the apparent change of thermal strategy is not clear from the results of this work.

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CHAPTER SIX

GENERAL DISCUSSION

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6.1 GENERAL DISCUSSION

The ultimate goal of research on a pest is to devise a strategy to keep the population below a level that is economically important. For a migrant pest like *Spodoptera exempta*, an essential component of control is the development of programmes to monitor movements of the pest and to predict the occurrence of outbreaks (Odiyo, 1979; Lambert, 1989). This requires knowledge about the factors that influence the movements of the species and its capacity to migrate. Previous work has indicated a connection between larval phase polyphenism and the population dynamics of *Spodoptera exempta* (Gatehouse, 1987; Gunn and Gatehouse, 1987; Woodrow *et al*. 1987; Gunn *et al*., 1989). Obtaining more information about phase polyphenism will clarify its role in relation to migration in this species.

This study has investigated the effects of phase polyphenism, in *Spodoptera exempta*, on larval development and thermoregulatory behaviour, feeding rates, food utilization and metabolic reserves in larvae, and reproduction in adults. The progress of experiments was affected by viral infection, which is a common problem with maintenance of lepidopteran cultures in the laboratory (eg Hill, 1991 with *Autographa gamma*; Colvin, 1990, with *Heliothis armigera*). For projects on tropical species in a temperate country like the United Kingdom, the delays in waiting for cultures to build up again after such infections, or for fresh supplies of insect material from the tropics can be considerable.

The results of the measurement of lipid concentration in *S. exempta* larvae (see Chapter 4) confirm the findings of Gunn and Gatehouse (1987) in

two (for Family 1 and the experiment on container effect) out of three cases examined. It is difficult to explain the entirely different results in the third case (Family 2), but it appears that more work is required to standardize densities, container types and sizes and numbers of larvae, that best bring out the differences between phases in the laboratory.

Regarding energy storage and utilization for flight, one area that requires study in *S. exempta* is the role of hormones. Fescemyer (1993) reported that adults from crowded larval cultures (black phase) of *Anticarsia gemmatalis* had lower "whole body carbohydrate and lipid weights" than those from uncrowded cultures (green phase). Though he also found these adults to have lower body weights, there is no indication that the fuel levels were adjusted for differences in body weight. If this was done, then Fescemyer's (1993) results contradict those of Gunn and Gatehouse (1987), who found that *gregaria* moths had higher lipid levels in *S. exempta*. However, Fescemyer (1993) also found that hyperlipemic responses (elevation of haemolymph lipid concentration in response to extracts of corpora cardiaca) of black phase adults were approximately two times greater than those of green phase adults. This is interpreted as an indication of greater capability, in the black phase, to mobilize lipids.

A similar investigation for *S. exempta* is required. In addition to the phase difference in lipid content (Gunn and Gatehouse, 1987; Chapter 4) there may also be differences in the ability of the phases to mobilize energy reserves for flight, as well as the allocation of reserves to other energy-demanding activities. In *A. gemmatalis*, black phase moths had

lower wing loadings which Fescemyer (1993) considered as one reason why they utilized less lipids and carbohydrates during flight than green phase moths. Larval crowding has been shown to influence wing loading in other Lepidoptera (Long, 1953; Iwao, 1962). Gunn and Gatehouse (1993) do not agree that there is any connection between wing loading and flight potential in *S. exempta* because earlier work by Parker and Gatehouse (1985) did not find any evidence in support. Instead, Gunn and Gatehouse (1993) found that *gregaria* moths selected for prolonged flight were heavier than non-selected ones. These reports indicate interspecific differences in life history strategy, but the role of hormones in the 'migratory syndrome' (Gunn and Gatehouse, 1993) of *S. exempta* still deserves study as does the hormonal basis of the phase transformation in the larval stage.

Knowledge of the effect of phase on larval food consumption and utilization is also necessary because nutrition is the source of energy and materials for other physiological processes (Slansky and Scriber, 1985; Hayes *et al.*, 1992). With the evidence indicating that variation in migratory potential depends on parallel variation in fuel reserves or in efficiency of their utilization (Fescemyer, 1993; Gunn and Gatehouse, 1993.), it is all the more important to investigate the processes leading to differences in fuel stores in the first place.

Data collection for the calculation of nutritional indices is time-consuming and tedious (Klein and Kogan, 1974; Scriber and Slansky, 1981). Several aspects of both plant and insect biology may affect the calculation of indices. Considerable care is therefore needed in the collection of these data,

and this requires the investment of even more time. Nevertheless, nutritional indices are valuable tools in the study of plant-insect interactions (Bowers *et al.*, 1991) and in comparing foraging strategies (Slansky, 1993).

In studying the effect of larval phase on nutrition, one of the main problems encountered was the use of grouped larvae. Bowers *et al.* (1991) found that solitary and grouped larvae of the buckmoth, *Hemileuca lucina* Hy. Ed. (Saturniidae), had similar growth rates on new leaves but differed significantly on mature leaves. Crowding may lead to competition for highest quality food (Stamp and Bowers, 1990). This competition may cause reduction in growth rates, and its intensity may be determined by the size of container used for rearing the larvae (Bowers *et al.*, 1991).

It is not possible now to estimate the extent to which the nutritional indices obtained for *solitaria* and *gregaria* in this project (Chapter 4) were affected by crowding per se rather than the physiological changes that phase causes. Attempts would have to be made, in future studies, to separate these effects as much as possible. It may be necessary to compare different container sizes and select one that is found most suitable. Bowers *et al.* (1991) recommend the use of grouped larvae for nutritional tests if the tendency to aggregate in the field is strong. In *S. exempta*, there is not much evidence of a tendency for the larvae to aggregate (Brown, 1962), but the presence of other larvae is normally necessary for the transformation to the *gregaria* phase. The ideal method for measuring consumption in the *gregaria* larvae is probably one that allows both data collection for

individual larvae and physical contact between such larvae and others. Such an experiment would be extremely difficult, if not impossible, to design.

Consumption rates have two major components: the proportion of time spent feeding and the speed of "biting and chewing" or the instantaneous feeding rate (Jones *et al.*, 1981; Bowdan, 1988; Slansky, 1993). The phases of *S. exempta* may differ in these "fine-scale measures" (Slansky, 1993) which could be considered for inclusion in further studies on this subject. The work of Simmonds and Blaney (1986) indicated a difference in the time spent feeding but the subject requires more detailed investigation.

In both the studies on adult fecundity (Chapter 3) and larval nutrition (Chapter 4), the differences between the results of experiments using first-generation and third-generation insects reared in the *solitaria* phase were difficult to explain. Further studies would need to consider whether the intensification of *solitaria* characters that this method (of rearing *solitaria* for a number of generations) seeks to achieve (Faure, 1943; Matthée, 1946), affects the extent of expression of *gregaria* characters.

Other authors have encountered similar problems in studying the effects of phase or population density on insects. In *Autographa gamma* (Zaher and Long, 1959) females from crowded cultures laid more eggs than *solitaria* in four out of five generations studied, but the difference was significant only in generation 1. In generation 4, the phase difference was reversed. This reversal was attributed to low temperature prevailing through that experiment and slowing down development, which conditions were considered more

suitable for egg production in solitary cultures (Long and Zaher, 1959).

Gu and Danthanarayana (1990) state that analysis of the influence of population density on insect populations require sophisticated techniques that can separate direct effects of density from indirect effects associated with increased number per unit area. The effect of population density is made so complex because populations interact with such a highly variable environment in nature.

Gruys (1970) also wrote: "... effects of crowding have been found in several insects. Expression is as variable as their occurrence in general. No frequently studied characteristic is similarly affected by crowding; every species seems to present a special case. Even within one species, fundamental differences occur between authors, between different stages and degrees of crowding".

From these statements, and from some of the results obtained in this work, it appears that many of the effects of phase polyphenism on physiology, behaviour and reproduction in *Spodoptera exempta* will require additional study before they are fully understood. The phases *solitaria* and *gregaria* have been applied on the basis of rearing density and colour differences. These criteria may, however, not be definite indicators of the extent of physiological changes associated. Larvae reared at the same density of the same colour may differ in physiology.

In spite of these difficulties in studying phase polyphenism, it is a subject worth pursuing considering its importance in relation to the temporal and spatial dynamics of this species and other migrant pests. More information on the role of phase is

likely, therefore, to be of value in designing appropriate programmes for the prediction and management of armyworm outbreaks.

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APPENDICES

APPENDIX TO CHAPTER THREE

AN(C)OVA TABLES ON WHICH TABLES 3.1-3.10 ARE BASED

3.1 Tests of Significance for FECUNDITY (g1)* using Covariate

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	21774422.14	294	74062.66		
REGRESSION	7849544.99	1	7849545.0	105.99	.000
DIET	2642342.51	1	2642342.5	35.68	.000
PH	411838.77	1	411838.77	5.56	.019
FAM	1880928.51	8	235116.06	3.17	.002
DIET BY PH	2396.67	1	2396.67	.03	.857
DIET BY FAM	580313.39	8	72539.17	.98	.452
PH BY FAM	1580271.16	8	197533.90	2.67	.008
DIET* PH*FAM	514927.54	8	64365.94	.87	.543
(Model)	15462563.54	36	429515.65	5.80	.000
(Total)	37236985.68	330	112839.35		

3.2 Tests of Significance for FECUNDITY (g3)* using Covariate

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	9247798.92	109	84842.19		
REGRESSION	593246.95	1	593246.95	6.99	.009
DIET	848123.95	1	848123.95	10.00	.002
PH	182665.03	1	182665.03	2.15	.145
FAM	307704.22	3	102568.07	1.21	.310
DIET BY PH	634747.66	1	634747.66	7.48	.007
DIET BY FAM	469147.20	3	156382.40	1.84	.144
PH BY FAM	403641.34	3	134547.11	1.59	.197
DIET*PH*FAM	948625.53	3	316208.51	3.73	.014
(Model)	4387901.88	16	274243.87	3.23	.000
(Total)	13635700.80	125	109085.61		

* g1 - experiment with first-generation *solitaria*;
g3 - experiment with third-generation *solitaria*.

3.3 Tests of Significance for NUMBER OF EGG BATCHES (g1) using Covariate

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	330.69	294	1.12		
REGRESSION	16.78	1	16.78	14.92	.000
DIET	127.86	1	127.86	113.67	.000
PH	.00	1	.00	.00	.951
FAM	27.00	8	3.38	3.00	.003
DIET BY PH	.01	1	.01	.01	.921
DIET BY FAM	6.52	8	.82	.72	.670
PH BY FAM	28.10	8	3.51	3.12	.002
DIET BY PH BY FAM	25.32	8	3.17	2.81	.005
(Model)	231.60	36	6.43	5.72	.000
(Total)	562.29	330	1.70		

3.4 Tests of Significance for NUMBER OF EGG BATCHES (g3)

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	363.13	110	3.30		
DIET	18.29	1	18.29	5.54	.020
PH	8.07	1	8.07	2.44	.121
FAM	21.85	3	7.28	2.21	.091
DIET BY PH	5.90	1	5.90	1.79	.184
DIET BY FAM	10.17	3	3.39	1.03	.384
PH BY FAM	8.44	3	2.81	.85	.469
DIET*PH*FAM	37.01	3	12.34	3.74	.013
(Model)	109.73	15	7.32	2.22	.010
(Total)	472.86	125	3.78		

3.5 Tests of Significance for OVIPOSITION PERIOD (g1) using Covariate

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	536.64	294	1.83		
REGRESSION	9.84	1	9.84	5.39	.021
DIET	180.00	1	180.00	98.61	.000
PH	.01	1	.01	.01	.931
FAM	52.90	8	6.61	3.62	.000
DIET BY PH	.16	1	.16	.09	.770
DIET BY FAM	13.25	8	1.66	.91	.511
PH BY FAM	35.66	8	4.46	2.44	.014
DIET BY PH BY FAM	41.33	8	5.17	2.83	.005
(Model)	333.14	36	9.25	5.07	.000
(Total)	869.78	330	2.64		

3.6 Tests of Significance for OVIPOSITION PERIOD (g3)

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	480.20	110	4.37		
DIET	32.51	1	32.51	7.45	.007
PH	10.44	1	10.44	2.39	.125
FAM	31.65	3	10.55	2.42	.070
DIET BY PH	4.33	1	4.33	.99	.321
DIET BY FAM	19.39	3	6.46	1.48	.224
PH BY FAM	17.64	3	5.88	1.35	.263
DIET BY PH BY FAM	31.28	3	10.43	2.39	.073
(Model)	147.23	15	9.82	2.25	.009
(Total)	627.43	125	5.02		

3.7 Tests of Significance for POP (g1)

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	284.18	291	.98		
DIET	2.51	1	2.51	2.57	.110
PH	12.06	1	12.06	12.35	.001
FAM	82.81	8	10.35	10.60	.000
DIET BY PH	.18	1	.18	.19	.665
DIET BY FAM	6.20	8	.78	.79	.609
PH BY FAM	14.38	8	1.80	1.84	.069
DIET BY PH BY FAM	8.93	8	1.12	1.14	.334
(Model)	127.08	35	3.63	3.72	.000
(Total)	411.25	326	1.26		

3.8 Tests of Significance for POP (g3)

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	249.68	109	2.29		
DIET	9.13	1	9.13	3.98	.048
PH	5.94	1	5.94	2.59	.110
FAM	37.05	3	12.35	5.39	.002
DIET BY PH	.09	1	.09	.04	.840
DIET BY FAM	11.20	3	3.73	1.63	.187
PH BY FAM	3.67	3	1.22	.53	.660
DIET BY PH BY FAM	6.97	3	2.32	1.01	.390
(Model)	74.05	15	4.94	2.16	.012
(Total)	323.73	124	2.61		

3.9 Tests of Significance for LONGEVITY (g1) using Covariate

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	1061.75	231	4.60		
REGRESSION	21.92	1	21.92	4.77	.030
DIET	701.72	1	701.72	152.67	.000
PH	1.50	1	1.50	.33	.568
FAM	2777.48	5	555.50	120.86	.000
DIET BY PH	26.18	1	26.18	5.70	.018
DIET BY FAM	90.27	5	18.05	3.93	.002
PH BY FAM	53.69	5	10.74	2.34	.043
DIET BY PH BY FAM	26.36	5	5.27	1.15	.336
(Model)	3699.12	24	154.13	33.53	.000
(Total)	4760.87	255	18.67		

3.10 Tests of Significance for LONGEVITY (g3)

Source of Variation	SS	DF	MS	F	Sig of F
WITHIN CELLS	719.18	106	6.78		
DIET	236.55	1	236.55	34.86	.000
PH	.12	1	.12	.02	.894
FAM	217.54	3	72.51	10.69	.000
DIET BY PH	1.84	1	1.84	.27	.604
DIET BY FAM	54.79	3	18.26	2.69	.050
PH BY FAM	42.28	3	14.09	2.08	.108
DIET BY PH BY FAM	11.48	3	3.83	.56	.640
(Model)	564.60	15	37.64	5.55	.000
(Total)	1283.78	121	10.61		

Appendix 3.11

Relationship between fecundity and weight of *Spodoptera exempta* moths.

I. with first-generation *solitaria*

II. with third-generation *solitaria*

sw = *solitaria* moths fed distilled water

ss = *solitaria* moths fed sucrose solution

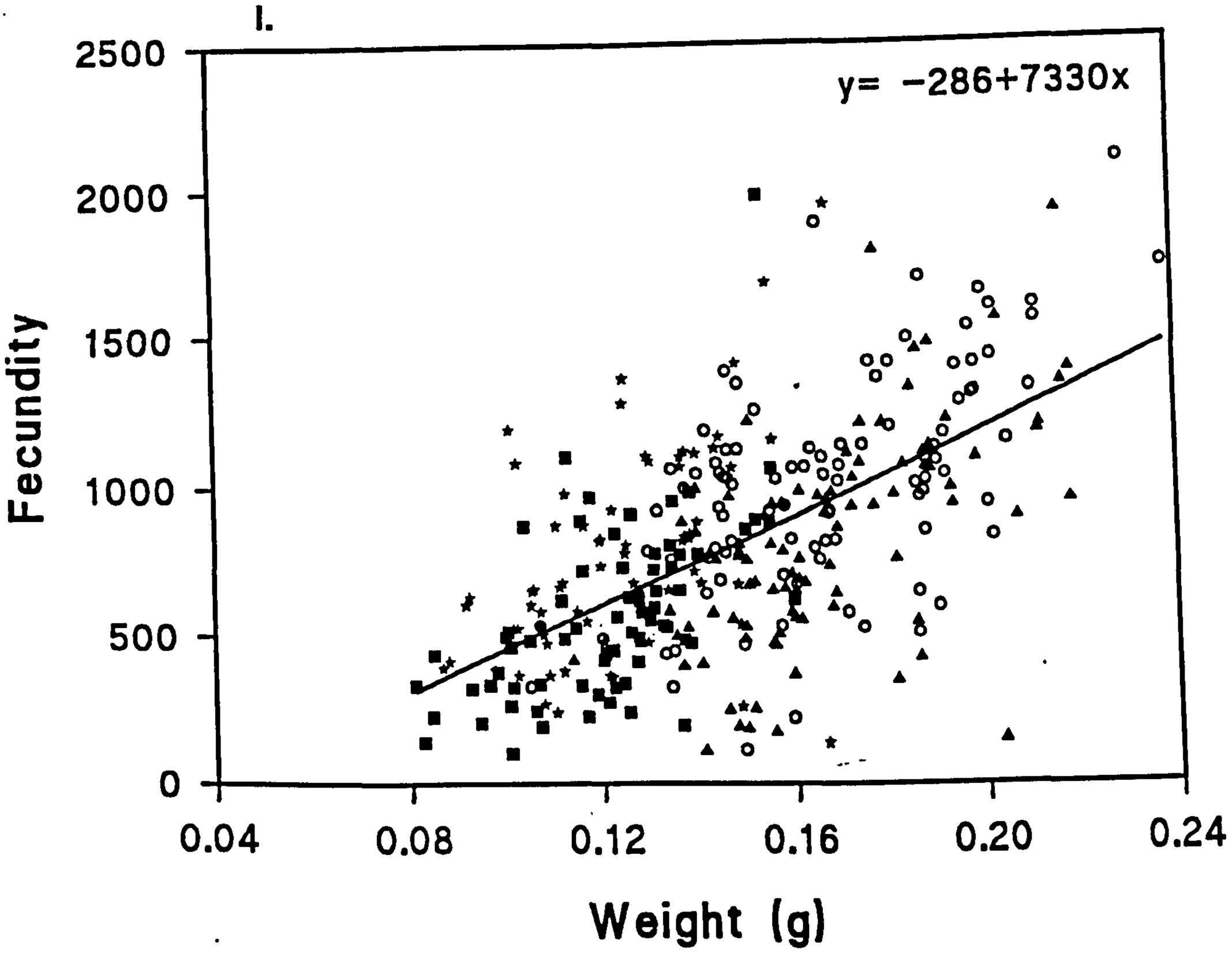
gw = *gregaria* moths fed distilled water

gs = *gregaria* moths fed sucrose solution

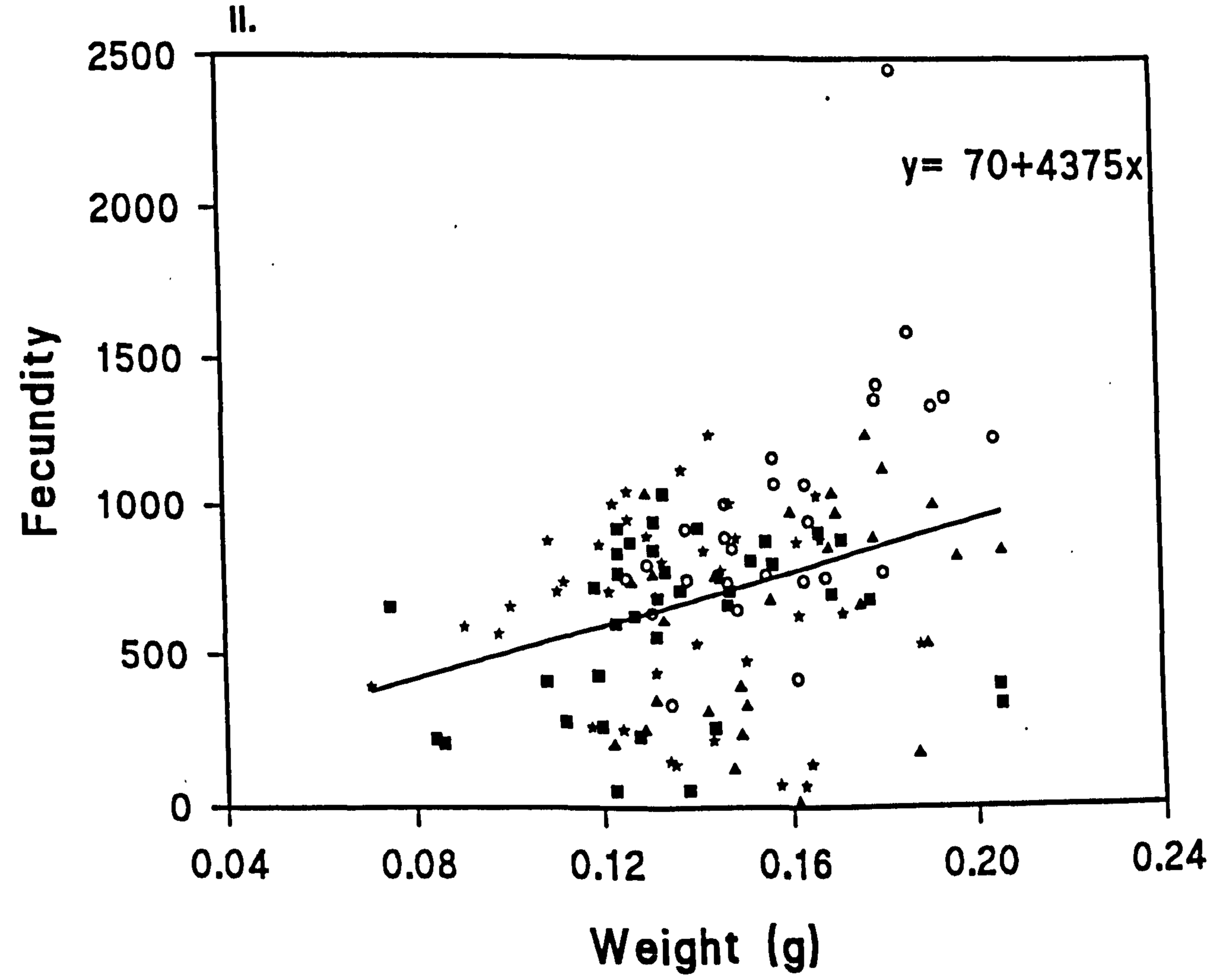
I. $r^2 = 0.369$, $P < 0.001$

II. $r^2 = 0.108$, $P < 0.001$

I.


y= −286+7330x
2500
2000
1500
1000
500
0
Fecundity
0.04
0.08
0.12
0.16
0.20
0.24
Weight (g)

II.


y= 70+4375x
2500
2000
1500
1000
500
0
Fecundity
0.04
0.08
0.12
0.16
0.20
0.24
Weight (g)

APPENDIX TO CHAPTER FOUR

Appendix 4.1

Method of analysing larvae for triglycerides

1. Freeze-dry larva
2. Break up larva by sonication if possible, or by an appropriate means (glass rod used in this experiment). Add 2 ml chloroform:methanol (2:1) and leave to extract in stoppered vial (glass centrifuge tube used in this project).
3. Centrifuge (1000 g x 2 min)
4. Take 100 μl supernatant and dry it down in a stream of nitrogen.
5. Add 200 μl distilled water
6. Add 500 μl ethanol
7. Add 1 drop potassium hydroxide (from Sigma kit)
8. Cover with parafilm
9. Heat tube at 65 °C for 20 min
10. Add 1000 μl magnesium sulphate (from Sigma kit)
11. Centrifuge (1000 g x 2 min)
12. Take 200 μl of the supernatant
13. Add 1000 μl of triglyceride reagent (from Sigma kit)
14. After 1-2 min read OD at 340 nm vs a distilled water blank
15. Add 4 l glycerokinase (from Sigma kit)
16. Read OD after 5 min.