A phylogenetic study of the Australian Heliothinae, and the cytogenetics of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae)

by

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Figure 1.1. Moths of the 15 taxa used for the phylogenetic analysis of the Heliothinae. (Photograph by John Green)

Key to photograph:

Helicoverpa punctigera *Helicoverpa armigera* *Helicoverpa assulta*

"Heliothis" rubrescens *Heliothis punctifera*

Heliocheilus eodora *Heliocheilus sp.1* *Heliocheilus clathrata*

Heliocheilus aberrans *Heliocheilus sp.2* *Heliocheilus moribunda*

Calophasidia angustula *Spodoptera litura*

Agrotis infusa *Mythimna convecta*

Statement of Authorship

The work described in this thesis, except where indicated by references or specific acknowledgements, is entirely my own.

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Abstract

Helicoverpa armigera and *H. punctigera* are the most economically damaging insect pests on cotton and other crops in Australia. Although much research has been done on the biology of these species (Zalucki et al. 1986), there are many gaps in the knowledge of their genetics, and their cytogenetics have not been studied. Details of their taxonomic relationships with other Heliothinae have been controversial.

A phylogenetic analysis of the relationship of these species with other Australian Heliothinae was done based on allozyme electrophoresis of the moths. From this study, this technique was found to be useful for establishing relationships within and between genera, but of little use at the level of subfamily, in the family Noctuidae. The phylogeny obtained is in agreement with that derived from the most recent morphological study of the Heliothinae (Matthews 1987a). The relative diversity within the Australian genera of the Heliothinae was established. The relationship of *Calophasidia angustula* (Stiriinae) to the Heliothinae is discussed.

Helicoverpa armigera and *H. punctigera* have very similar karyotypes, with 31 pairs of chromosomes in a graded series of sizes. No pairs could be differentiated, by a variety of techniques. Cytological evidence suggests that female meiosis is achiasmatic. The sex chromosomes are XY in the female and XX in the male, with sex chromatin bodies present only in the female somatic interphase nuclei. These features are typical of the Noctuidae. Precocious separation of the bivalents into univalents at metaphase I was observed in some spermatocytes of *H. punctigera*. This species also had a consistently greater number of bivalents with distal chiasmata in each spermatocyte at male metaphase I than *H. armigera*.

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General Introduction

The moths *Helicoverpa armigera* and *H. punctigera* are the most economically damaging insect pests in Australia, affecting a wide range of agricultural and horticultural crops, and are also found on many other exotic and native plant species. These moths are highly fecund, have several generations each year, have facultative diapause, and can move long distances as adults, and thus can rapidly exploit crops, particularly monocultures. Most of the economic loss is caused by the larvae feeding on the fruits and seeds of cotton, soybeans, chick-pea and pigeon pea, and the leaves of tobacco. *H. armigera* has also developed some resistance to most groups of insecticides used to control it, including DDT and endosulfan (Kay et al. 1983), and synthetic pyrethroids (Gunning et al. 1984). The cost of *Helicoverpa* species in Queensland crops in the year 1988-89 was estimated at $73.3 million, including the costs of insecticidal control, and of lost production (Mc Gahan et al. 1990). The cost to the whole of Australia may be three times this.

As control of these species by insecticides becomes more difficult and expensive, due to the development of resistance, and more environmentally unacceptable, alternative means of control are being investigated. Much research effort is now being directed to the development of biological control agents. The nuclear polyhedrosis virus (NPV) and the endotoxin of the bacterium *Bacillus thuringiensis* (Bt) are both effective against *Helicoverpa* species, and research includes the genetic engineering of more potent and stable strains of NPV and Bt, and the incorporation of Bt endotoxin production into the genome of host plants such as cotton (King and Coleman 1989). With the prospect of releasing such agents to control the *Helicoverpa* species, it is essential to understand the poorly resolved taxonomy of these and other closely related species which may also be affected.

This thesis is divided into two parts. In the first part, the taxonomy of the Australian Heliothinae, to which *Helicoverpa* belongs, is examined by phylogenetic analyses based on allozyme electrophoresis. Allozyme electrophoresis is a relatively reliable, quick and inexpensive method, which uses variations in allozymes as phenotypic markers, and is

useful for studies in population genetics and taxonomy. Although there has been much work done on the biology of *H. armigera* and *H. punctigera* (Zalucki et al. 1986), this has never been placed in the context of the whole subfamily, the Heliothinae.

Part 2 of this thesis focuses on the cytogenetics of *H. armigera* and *H. punctigera.* The karyotype of both species is examined, and female meiosis is observed to see if it is achiasmatic. Alternative characters for taxonomic study may come from cytogenetic observations. The number, size and shape of the chromosomes could be useful characters for taxonomic comparisons.

There is only limited knowledge of the genetics of these *Helicoverpa* species, and most of this concerns the genetics of insecticide resistance in *H. armigera* (e.g. Daly et al. 1988). Further genetic research is impeded by the lack of linkage maps, the construction of which, due to the large number of chromosomes in *Helicoverpa* species, would only be practical if female meiosis proved to be achiasmatic, as has been demonstrated for some other Lepidoptera. Such maps would be useful for studying linkage between resistance to different pesticides or biological control agents.

Part 1: A phylogenetic study of the Australian Heliothinae, based on allozyme electrophoresis.

1.1 Introduction

The subfamily Heliothinae in the family Noctuidae includes about 400 species of moths, mainly found in semi-desert, scrub and savannah in the seasonally arid tropics and subtropics of Australia, Africa, Asia and North America (Matthews 1987a). Most species are polyphagous, and the larvae feed mainly on the flowers, fruits and seed of their host-plants, usually annuals and low-growing perennials. Although some Heliothinae are among the world's most serious economic pests, many species are of no economic importance. It is essential to understand the systematics of the Heliothinae, not only to aid the study of the economically damaging species, but also to conserve the non-pest species once biological control methods become important for pest management.

1.1.1 The Australian Heliothinae

This study covers 11 species of Heliothinae, representing 4 of the 5 Australian genera (Nielsen et al. in prep). The only Australian genus not included is *Adisura*, which has one described species *A. litarga* (Turner)†. The 11 species in this study include all the described Australian species of *Helicoverpa*, *Heliothis* and "*Heliothis*" (previously included in *Heliothis*) except the uncommon *Helicoverpa prepodes* (Common), *Heliothis roseivena* (Walker) and "*Heliothis*" *tertius* (Roepke). Six species of *Heliocheilus* including 2 undescribed species are also included in this study. Of the 41 described Australian Heliothinae, 32 are in the genus *Heliocheilus*. The current classification for the Heliothinae studied, based on morphology, as well as the 4 species from other

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† In Part 1, the author of each species and year of publication is given in Table 1.1 for the species included in this study, and the author is given following the first mention of all other species referred to. This information is not included for species referred to in Part 2, which is not a taxonomic study, since such information was not given in most of the cited publications and the identity of the species could not be verified.

Table 1.1. Classification of the species of Noctuidae (Nielsen et al. in prep) included in this study.

Lepidoptera

Noctuidae

Heliothinae

Helicoverpa punctigera (Wallengren, 1860)

Helicoverpa armigera (Hübner, 1805)

Helicoverpa assulta (Guenée, 1952)

"Heliothis" rubrescens (Walker, 1858)

Heliothis punctifera Walker, 1857

Heliocheilus eodora (Meyrick, 1902)

Heliocheilus sp.1 (undescribed)

Heliocheilus clathrata (Warren, 1913)

Heliocheilus aberrans (Butler, 1886)

Heliocheilus sp.2 (undescribed)

Heliocheilus moribunda (Guenée, 1852)

Stiriinae

Calophasidia angustula (Turner, 1941)

Amphipyrinae

Spodoptera litura (Fabricius, 1775)

Hadeninae

Mythimna convecta (Walker, 1857)

Noctuinae

Agrotis infusa (Boisduval, 1832)

subfamilies included for outgroup analysis, is given in Table 1.1, and the moths are shown in Fig. 1.1.

1.1.2 Genera of the Heliothinae in this study

Helicoverpa Hardwick, 1965

Helicoverpa is now a well defined group (Matthews 1987a), but its taxonomy has been controversial in the past. *H. punctigera* was confused with *H. armigera* until revised by Common (1953). The separation of *H. armigera*, *H. punctigera* and *H. assulta* to a new genus *Helicoverpa*, previously a subgenus of *Heliothis*, was suggested by Hardwick (1965), but this has only recently been accepted in Australia.

Helicoverpa armigera and *H. punctigera* are both serious pests on crops in Australia. Both species are highly polyphagous; Zalucki et al. (1986) recorded 159 host-plant species in 49 plant families for either species. *H. punctigera* is found mainly on dicotyledonous plants, whereas *H. armigera* occurs on both dicotyledons and monocotyledons. Although most of the economic loss caused by these species is on cotton, soybean, chick-pea, pigeon pea and tobacco, many other crops suffer significant damage, including maize, sorghum, sunflower, safflower, lucerne, tomatoes and cut flowers (Fitt 1989).

H. armigera occurs in Africa, the Middle East, southern Europe, and parts of Asia, Australia and the Pacific, and *H. punctigera* is found only in Australia. *Helicoverpa assulta* occurs in Australia, southern Asia and Africa, and is a pest on crops including tobacco and Cape gooseberry (*Physalis peruviana*, Solonaceae). *H. prepodes* is known from only about 20 specimens from southern Australia (Common 1985). *H. zea* (Boddie) is a major pest on agricultural crops in the Americas. These species have been widely referred to under their former generic name *Heliothis*.

"*Heliothis*" s. lat.

Matthews (1987a) gave much evidence that "*Heliothis*" *rubrescens*, along with "*H*"

tertius, known from only 2 specimens, should be assigned to a separate genus from *Heliothis*, morphologically intermediate between *Heliothis* and *Helicoverpa*. Both these species, endemic to the Australian region, have not been recorded as pests.

Heliothis Ochsenheimer, 1816

Heliothis punctifera is the only Australian member of the genus, which includes a major North American pest *H. virescens* (Fabricius).The evidence presently available makes this a paraphyletic group, if *Heliocheilus* is recognised as a distinct genus (Matthews 1987a).

Heliocheilus Grote, 1865

Hardwick (1970) placed *Heliocheilus* as a subgenus of *Heliothis*, but Matthews (1987b) recognised *Heliocheilus* and *Heliothis* as distinct genera. For the species where host-plant records exist, they have only been recorded from grasses. This genus is cosmopolitan, but is most diverse in Australia, where 32 endemic species have been described (Nielsen et al. in prep). One African species, *H. albipunctella* (de Joannis), has recently developed pest status on millet (Deeming 1978), but no others in this genus, including the Australian species, are considered to be pests.

1.1.3 The relationship of the Stiriinae to the Heliothinae

"That the higher classification of the Noctuidae is in disarray and in need of extensive reevaluation is probably the only point on which all workers in the field agree." (Kitching 1984).

Until recently, it was thought that the Stiriinae occurred only in North America, but a few species outside this region have recently been recognised as Stiriinae, from South America, Africa and the Palaearctic (Matthews 1987a). Two genera from Australia, known only from adult specimens, have been classified as Stiriinae (Nielsen et al. in prep). They occur mainly in semi-arid regions in habitats similar to those occupied by the Heliothinae (see section 1.1).

Historically, the Stiriinae have been linked with a variety of subfamilies of the Noctuidae (Kitching 1984), and their classification is still not resolved. Most recently, the Stiriinae have been tentatively placed as a sister group to the Heliothinae. There are good adult characters that distinguish between these subfamilies, including a character of the male sternum, and a feature of the hind wing venation (Matthews 1987a). The best evidence for the monophyly of the Stiriinae is the form of the spinneret in larvae (Matthews 1987a) however comparatively few of the larvae are known. The Heliothinae are well defined as a monophyletic group by the spiny skin, and a character of the setae, in larvae (Matthews 1987a), and by adult male genitalia characters (Hardwick 1970).

The most frequently cited characters that link these subfamilies is that the larvae of both taxa feed on the flowers and seeds of their host plants, and that they occur in similar habitats (Hardwick 1970, Matthews 1987a). Both authors acknowledge however, that the larval stages of most Stiriinae are unknown. There are also some similarities of the male and female genitalia. Both the Stiriinae and the Heliothinae have affinities with a possibly intermediate group, the "Pyrrhia group". Matthews (1987a) could not find evidence to define whether the "Pyrrhia group" are monophyletic, and thus could not resolve the relationship between the Stiriinae and the Heliothinae.

1.1.4 Aims of this taxonomic study

A taxonomic analysis was made of the relationships between 11 Heliothinae species based on allozyme electrophoresis, independent of any morphological taxonomy. The broad aim was to see what agreement there was between this phylogenetic analysis of electrophoretic data and the traditional morphological taxonomies. More particularly, several aspects of the taxonomy of the Heliothinae, not well resolved by morphological taxonomy, were addressed using the phylogenetic analysis of electrophoretic data:

1. What are the relationships within and between the genera *Helicoverpa*, "*Heliothis*" and *Heliothis*, which include all the major pest species of the Heliothinae, as well as some non-pest species?
2. What is the relationship between the large genus *Heliocheilus*, with many Australian

species, and the other Australian Heliothinae genera?

3. What are the levels of diversity among and between the Heliothinae genera?

4. How closely related are the Heliothinae and the Stiriinae?

In addition it was asked; how useful is allozyme electrophoresis as a taxonomic tool for the study of the Noctuidae, and particularly the Heliothinae?

1.1.5 Phylogenetic analysis

There are numerous methods for deriving phylogenetic trees, none of which is completely suitable for inferring phylogenies from allozyme frequency data. The most suitable methods, chosen for this study, are those that are constrained by a minimum of assumptions. None of these chosen methods make the assumption of constant rates of evolution for all taxa. Such an assumption is often unacceptable for electrophoretic data, where studies have shown that rates of change of electrophoretic characters are sometimes variable (Baverstock et al. 1979). By using several methods, phylogenetic relationships that are well supported by the data should be represented similarly by all methods. Less well resolved relationships would be expected to vary between and within methods.

It has been argued that the detailed use of allele frequencies offers little additional information to that derived from the presence or absence of alleles, because at the subspecies level or higher, entirely separate sets of alleles may be present (Farris 1981). However, the data used in this study revealed that within the Heliothinae, many alleles are shared between species within each genus. In particular in some of the *Heliocheilus* species, the main interspecific differences involved allele frequencies.

1.2 Materials and methods

1.2.1 Sample collections

The electrophoretic study was performed on adult moths, since this is the only life stage at which Lepidoptera can be readily identified morphologically and because it was the most convenient life stage to collect. The localities, dates and life stage collected are

Table 1.2a. Locations, dates and life stage of Noctuidae collected for electrophoretic analysis, shown in Fig. 1.2.

No.	Location	Date	Life stage	Collected by[a]
1	Quilpie, Qld	4.9.89	adults	JF,GF,PG
2	Windorah, Qld	5-8.9.89	adults	JF,GF,PG
3	Durham Downs, Qld	9-10.9.89	adults	JF,GF,PG
4	Myall Vale, NSW	winter.89	pupae	GF
5	246 km N of Yunta, SA	28.6.89	larvae	GF
6	Giralang, Canberra, ACT	11.11-4.12.89	adults	JF
7	Myall Vale, NSW	5-15.1.90	adults	JF
8	Within 40 km of Charleville,Qld	13-19.3.90	adults	JF,EE
9	30 km N of Cunnamulla, Qld	20-21.3.90	adults	JF,EE
10	Toowoomba, Qld	19.4.90	larvae	KR

[a]Collected by, JF: Jenny Fisk, GF: Gary Fitt, PG: Peter Gregg, EE: Ted Edwards, KR: Kerry Rynne.

Table 1.2b. Locality numbers (from Table 1.2a and Fig. 1.2) for specimens used for electrophoretic analysis.

Species	Localities	Species	Localities
Helicoverpa punctigera	2, 3, 6, 8	*Heliocheilus aberrans*	8
Helicoverpa armigera	4, 8	*Heliocheilus sp.2*	8
Helicoverpa assulta	10	*Heliocheilus moribunda*	8, 9
"Heliothis" rubrescens	1, 2, 3, 8	*Calophasidia angustula*	8
Heliothis punctifera	2, 3, 5, 8	*Spodoptera litura*	7
Heliocheilus eodora	8	*Agrotis infusa*	6
Heliocheilus sp.1	8	*Mythimna convecta*	6, 7
Heliocheilus clathrata	8, 9		

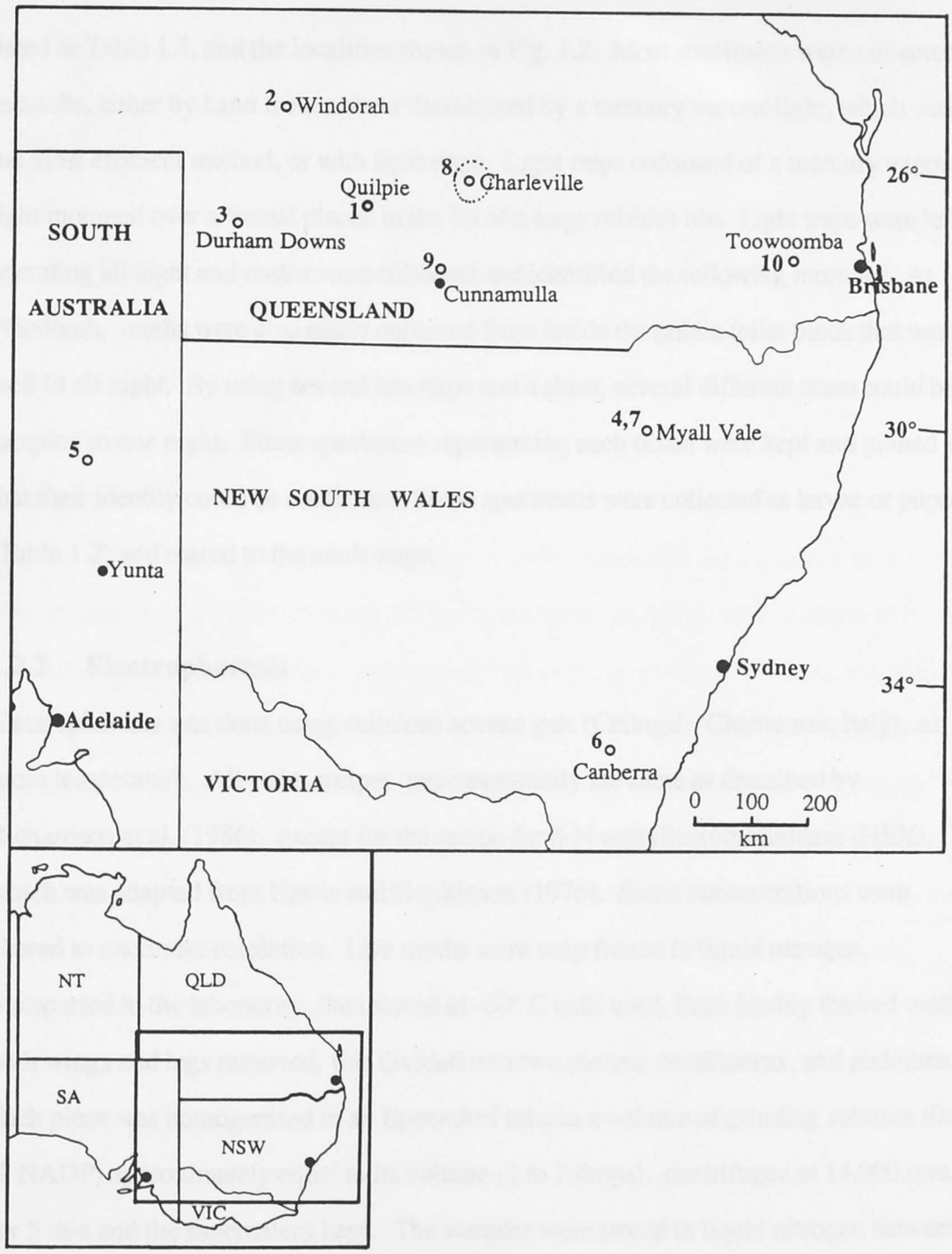

Figure 1.2. Map of eastern Australia with Noctuidae collection site locations (open circles) listed in Table 1.2.

listed in Table 1.2, and the localities shown in Fig. 1.2. Most specimens were collected as adults, either by hand from a sheet illuminated by a mercury vapour light, which was the most efficient method, or with light traps. Light traps consisted of a mercury vapour light mounted over a funnel placed in the lid of a large rubbish bin. Light traps were left operating all night and moths were collected and identified the following morning. At Windorah, moths were also easily collected from inside the public toilet block that was well lit all night. By using several bin traps and a sheet, several different areas could be sampled in one night. Some specimens representing each taxon were kept and pinned so that their identity could be confirmed. Some specimens were collected as larvae or pupae (Table 1.2) and reared to the adult stage.

1.2.2 Electrophoresis

Electrophoresis was done using cellulose acetate gels (Cellogel: Chemetron, Italy), at room temperature. All stain recipes were essentially the same as described by Richardson et al. (1986), except for the recipe for β-N-acetylhexosaminidase (HEX), which was adapted from Harris and Hopkinson (1976). Some concentrations were altered to maximise resolution. Live moths were snap frozen in liquid nitrogen, transported to the laboratory, then stored at -60° C until used. Each freshly thawed moth, with wings and legs removed, was divided into two pieces: head/thorax, and abdomen. Each piece was homogenised in an Eppendorf tube in a volume of grinding solution (0.01 % NADP) approximately equal to its volume (2 to 7 drops), centrifuged at 14,000 rpm for 5 min and the supernatent kept. The samples were stored in liquid nitrogen between runs.

Following the recommendations listed by Richardson et al. (1986), the following nomenclature has been used: Enzymes are referred to by their E. C. (Enzyme Commission) trivial name or upper-case abbreviation, and number (e.g. isocitrate dehydrogenase, IDH, 1.1.1.42). Enzyme loci are shown by an italic, lower-case version of the enzyme abbreviation (e.g. *Idh*). Where two isozymes were present for one enzyme, the faster migrating one was called -1 and the slower, -2 (e.g. IDH-1 and

IDH-2). Each allozyme was named A,B,C etc. in order of mobility, with A the fastest migrating allozyme.

Allozymes from the moth samples were resolved successfully with 24 enzyme systems. A single locus was represented by 16 of these, and two loci with each of the other 8, giving a total of 32 presumptive loci for comparison. For some enzymes, different parts of the moth or different running conditions were needed to resolve the different isozymes (Table 1.3).

Several other enzymes were investigated, but were not suitable for the moths in this study: Esterase (EST, 3.1.1.1) stains clearly using larvae, but is not visualised at all with adults on Cellogel. When gels were stained for hexokinase (HK, 2.7.1.1), for most species the bands of two isozymes overlapped. One of these isozymes was identical to the faster adenylate kinase isozyme (AK-1). Of the two AK isozymes, the faster may indeed be hexokinase. However they have been named AK for this study, and the HK isozymes were not used. Superoxidase dismutase (SOD, 1.15.1.1) could be resolved for only a few species, and so was not used. Guanine deaminase (GDA, 3.5.4.3) resolved with multiple bands that could not be interpreted. The bands for pyruvate kinase (PK, 2.7.1.40) could also not be interpreted. No activity was detected for purine nucleoside phosphorylase (NP, 2.4.2.1) or acid phosphatase (ACP, 3.1.3.2) in moths. The choice of enzyme systems used for this study was essentially random, based on the success of resolving and interpreting allozymes for the majority of taxa.

No enzyme stain was produced with 3 of the species for different isozymes: *Calophasidia angustula* for PGK, *Spodoptera litura* for GOT-2, and *Agrotis infusa* for PEP.A-2. For the data analyses that included these species, missing data could not be accepted by the programs, so the most common allozyme was assigned to the above species for the locus at which they did not stain. This was done to be consistent with allozyme data as a whole, which is conservative for the estimation of differences. Only a small proportion of amino-acid substitutions are electrophoretically detectable (Richardson et al. 1986).

Table 1.3. Electrophoretic running conditions and sample type for enzymes at 32 presumptive loci.

Enzyme	Enzyme name	EC #	Tissue[a]	Buffer[b]	Time[c]
ACON-1	aconitate hydratase	4.2.1.3	A	2	90
ACON-2			A	2	90
AK-1	adenylate kinase	2.7.4.3	A	4	30
AK-2			HT	4	40
ALD	aldolase	4.1.2.13	A	1	35
AO	aldehyde oxidase	1.2.3.1	A	2	90
ENOL	enolase	4.2.1.11	HT	3	30
FUM	fumarate hydratase	4.2.1.2	HT	1	35
GAPD	glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	HT	4	30
GOT-1	glutamate-oxaloacetate transaminase	2.6.1.1	HT	1	35
GOT-2			HT	1	35
αGPD	glycerol-3-phosphate dehydrogenase	1.1.1.8	HT	1	25
G6PD	glucose-6-phosphate dehydrogenase	1.1.1.49	A	1	40
GPI	glucose-phosphate isomerase	5.3.1.9	HT	3	30
HBDH	β-hydroxybutyrate dehydrogenase	1.1.1.30	A	1	60
HEX-1	β-N-acetylhexosaminidase	3.2.1.30	A	1	35
HEX-2			A	1	35
IDH-1	isocitrate dehydrogenase	1.1.1.42	HT	4	40
IDH-2			HT	4	40
LDH	lactate dehydrogenase	1.1.1.27	HT	1	35
MDH-1	malate dehydrogenase	1.1.1.37	HT	1	45
MDH-2			A	2	95
ME	malic enzyme	1.1.1.40	HT	1	30
MPI	mannose-phosphate isomerase	5.3.1.8	HT	3	20
PEP.A-1	peptidase A (val-leu)	3.4.11	A	5	35
PEP.A-2			A	5	35
PEP.B-1	peptidase B (leu-gly-gly)	3.4.11	A	5	35
PEP.B-2			A	5	35
PEP.D	peptidase D (phe-pro)	3.4.11	A	5	30
PGD	6-phosphogluconate dehydrogenase	1.1.1.44	HT	1	60
PGK	phosphoglycerate kinase	2.7.2.3	A	1	35
PGM	phosphoglucomutase	2.7.5.1	HT	3	30

[a] Tissue: A = abdomen. HT = head and thorax.

[b] Electrophoretic running buffers and voltage:
1: 20 mM Phosphate, pH 7.1 at 200 V.
2: 100 mM Phosphate, pH 7.1 at 60 V.
3: 15 mM Tris EDTA Maleate, pH 7.8 at 250 V.
4: 50 mM Tris Maleate, pH 7.8 at 200 V.
5: 50 mM Tris Citrate, pH 8.2 at 200 V.

[c] Time: Running time (minutes) at room temperature with bridges 6 cm apart.

1.2.3 Data analysis

The computer program Biosys-1, release 1.7 (Swofford and Selander 1989) was used to calculate: allele frequencies from genotype frequency data, the average number of alleles per locus, and the percentage of polymorphic loci for each taxon. Two measures of genetic distance between each pair of taxa were calculated (Nei's distance and the arc distance of Cavalli-Sforza and Edwards), as well as the percentage of fixed differences, and 4 different methods of phylogenetic analysis were used.

Nei's (1978) distances were calculated using the Biosys-1 program, for 15 taxa at 32 loci, for an initial assessment of the usefulness of the outgroup taxa, and for comparisons with other studies. Three of the possible outgroup taxa and 5 loci were not used in subsequent analyses (see section 1.3.1)

The arc distance of Cavalli-Sforza and Edwards (1967) was chosen as the distance measure for phylogenetic analysis using the distance Wagner method and the Fitch-Margoliash method. The arc distances were calculated, using the Biosys-1 program, for 12 taxa and 27 loci. This measure meets all the requirements for a genetic distance measure (Wright 1978): the measure is zero where the two populations have identical gene frequencies and unity if there are no alleles in common, irrespective of the number of alleles; the sum of the measures for component distances equals the total; the scale of the distance is that of the angular transformation of the frequencies.

In calculating the Cavalli-Sforza and Edwards arc distances, the transformed frequencies produce a scale that is stretched symmetrically near the extremes, but condensed in the middle, similar to the usual effects of factors on a percentage scale, and is greatly superior to untransformed scales, particularly for cases with more than two alleles (Wright 1978). This distance measure is not constrained by assumptions about constant rates of evolution, as are measures such as that of Nei (1972). Nei's distance is also unsuitable because it does not satisfy the triangle inequality (for distances among three taxa A, B

and C, the distance (A,C) must be less than or equal to the distance (A,B) plus the distance (B,C)).

The distance Wagner procedure (Farris 1972) was used with the computer program Biosys-1. The analysis, using Cavalli-Sforza and Edwards arc distances, was done using the multiple addition criterion algorithm of Swofford (1981). The method aims to find the tree with the minimum length. This is the most 'parsimonious' tree, representing a phylogeny in which the observed characters have evolved with the least change.

The maximum likelihood method of Felsenstein (1981) and the Fitch and Margoliash method (1967), were used with the computer program Phylip, version 3.2 (Felsenstein 1989). The maximum likelihood method is a statistical inference method that operates on character state data, and involves finding the tree that maximises the probability of having obtained the observed data. The assumptions made for this method are that the taxa evolve independently, each gene frequency changes by genetic drift, and the loci drift independently. These are reasonable assumptions for the taxa in this study which are known to be distinct species. With the large number of chromosomes in Lepidoptera, 31 pairs being the most common number for Noctuidae, it is reasonable to assume most loci will be independent.

The Fitch-Margoliash method, used in this study with arc distances of Cavalli-Sforza and Edwards, aims to minimise the "percent standard deviation", a measure of how well the inferred phylogeny fits the input data (the distances). Branch lengths of the tree are not constrained by an assumption of equal rates of evolution for all taxa. Both the maximum likelihood analysis and the Fitch-Margoliash analysis were performed using the "global branch swapping" and "jumble" options of the Phylip program to find the best trees.

The Hennig86 program for phylogenetic analysis, version 1.5 (Farris 1988) is not suitable for the analysis of gene frequency data unless the data are recoded. The data

were recoded in two ways. Firstly, the most common allele at each locus was selected, and each taxon was coded for the presence (1) or absence (0) of this allele. Due to the sample sizes tested at each locus, the lowest frequency recorded for the presence of an allele was 0.100. However, where there are very few fixed differences between taxa, and most differences involve only frequency differences, presence-absence coding may not be satisfactory, since the information from frequency changes would be lost. To take into account gene frequency differences, a second coding method was used. Loci from each taxon were coded (1) if the most common allele was present at a frequency of more than or equal to 0.500, and (0) if the frequency was less than 0.500.

All the analyses were done using an outgroup, so that evolutionary direction is given to the tree. The use of an outgroup assumes monophyly between ingroup taxa, which is well established for the Heliothinae by morphological taxonomy. It is assumed that traits shared between the outgroup and the ingroup are primitive. The accuracy of the placement of the root can be tested by using alternative outgroups, and seeing if the same rooting point is found with each different outgroup (Buth 1984).

The species selected for outgroup analysis were members of other subfamilies that were considered to be most closely related to, and monophyletic with, the Heliothinae, by Kitching (1984) in his review of the Noctuidae literature. It was anticipated that some, if not all of these species, would share sufficient allozyme markers with the Heliothinae, but still be distant enough to be useful for outgroup analysis. *Calophasidia angustula* was the only member of the controversial Stiriinae that could be collected. *Spodoptera litura* (Amphipyrinae), *Mythimna convecta* (Hadeninae) and *Agrotis infusa* (Noctuinae) are all common, widely distributed species, and were easily collected.

1.3 Results

1.3.1 Genetic variation

The allele frequencies for all 15 taxa at the 32 loci examined are given in Appendix 1, as well as the number of individuals sampled at each locus. Measures of genetic variation

Table 1.4. Genetic variation in 15 species at 32 loci. Standard errors for mean sample size/locus and mean no. of alleles/locus were ≤ 0.2 for all species.

Species	Mean sample size/locus	Mean no. of alleles/locus	% loci polymorphic
Helicoverpa punctigera	4.3	1.5	41
Helicoverpa armigera	4.1	1.3	25
Helicoverpa assulta	4.2	1.7	44
Heliothis punctifera	3.8	1.4	31
"Heliothis" rubrescens	3.8	1.5	41
Heliocheilus eodora	3.9	1.3	28
Heliocheilus sp.1	3.9	1.5	38
Heliocheilus clathrata	3.9	1.4	31
Heliocheilus aberrans	3.9	1.6	41
Heliocheilus sp.2	3.9	1.5	38
Heliocheilus moribunda	3.8	1.7	44
Calophasidia angustula	4.5	1.4	34
Spodoptera litura	3.8	1.3	19
Agrotis infusa	3.8	1.7	44
Mythimna convecta	3.9	1.4	34

within taxa are given in Table 1.4. The percentage of polymorphic loci as observed by allozyme electrophoresis ranged from 19% for *Spodoptera litura*, to 44% for *Heliocheilus moribunda* and *Agrotis infusa*. The average number of alleles per locus ranged from 1.3 to 1.7. An average of 4 moths were sampled at each locus. The mean number sampled per locus for each taxon is given in Table 1.4.

The percent fixed differences between species (Table 1.5) were as low as 0% to 16% within the *Heliocheilus* species, 25% to 31% within *Helicoverpa* species and up to 59% within all ingroup species. Percent fixed differences between each outgroup taxon and all the ingroup taxa ranged from 48% to 61% for *Spodoptera litura*, 54% to 65% for *Agrotis infusa*, 59% to 81% for *Mythimna convecta* and 68% to 87% for *Calophasidia angustula*.

After examining the Nei distance matrix (Table 1.5), *Spodoptera litura* was selected as the most suitable outgroup. The other three possible outgroup species, *Calophasidia angustula*, *Agrotis infusa* and *Mythimna convecta* all had distances to the ingroup taxa greater than 1.2, which was considered by Richardson et al. (1986) to be the limit of usefulness. At Nei's distance of 1.2, which corresponds to about 60-70% fixed differences, many of the similarities may be due to chance convergence (Richardson et al. 1986). Nei distances within the ingroup species ranged from 0.045 between *Heliocheilus eodora* and *H. sp.2*, up to 1.100 between *Helicoverpa armigera* and *Heliocheilus clathrata*. Despite their large genetic distance from the ingroup taxa, *C. angustula* and *A. infusa*, when used as outgroups, also rooted the trees to the same point as did *S. litura*, confirming the validity of the placement of the root.

It is worth noting that for the loci at which *Calophasidia angustula* had alleles unique among the taxa studied, the mobility differences of the allozymes were consistently much greater than between the other outgroup taxa and the ingroup taxa. Examples of this were at the loci *Hex-1*, *Ao*, *Pgd*, *Mpi*, *Me*, *Ldh* and *G6pd*. Another feature unique to *C. angustula* was that on gels for which the allozymes were visualised with ultra violet light (HEX, PGK and ALD), a pink fluorescent band was always present close to the origin.

Table 1.5. Matrix of Nei (1978) unbiased genetic distance coefficients (above diagonal) and percent fixed differences (below diagonal) for 15 taxa. Abbreviations for genera: *HV: Helicoverpa, HT: Heliothis, "H": "Heliothis", HH: Heliocheilus, CA: Calophasidia, SP: Spodoptera, AG: Agrotis, MY: Mythimna.*

	Species	1	2	3	4	5	6	7	8
1	*HV punctigera*	*****	.474	.539	.662	.685	.724	.777	.908
2	*HV armigera*	31.3	*****	.410	.530	.656	.918	.972	1.100
3	*HV assulta*	28.1	25.0	*****	.448	.546	.617	.699	.936
4	*HT punctifera*	37.5	37.5	31.3	*****	.531	.558	.611	.699
5	*"H" rubrescens*	25.0	37.5	31.3	31.3	*****	.870	.904	.908
6	*HH eodora*	34.4	53.1	31.3	28.1	43.8	*****	.088	.191
7	*HH sp.1*	37.5	53.1	40.6	34.4	43.8	3.1	*****	.211
8	*HH clathrata*	46.9	59.4	43.8	34.4	43.8	9.4	9.4	*****
9	*HH aberrans*	40.6	50.0	40.6	37.5	43.8	9.4	6.3	15.6
10	*HH sp.2*	34.4	56.3	34.4	25.0	43.8	0.0	6.3	3.1
11	*HH moribunda*	40.6	53.1	34.4	31.3	40.6	6.3	6.3	9.4
12	*CA angustula*	77.4	87.1	80.6	77.4	71.0	71.0	74.2	67.7
13	*SP litura*	54.8	48.4	51.6	51.6	58.1	61.3	51.6	58.1
14	*AG infusa*	61.3	64.5	54.8	61.3	64.5	64.5	58.1	58.1
15	*MY convecta*	68.8	68.8	59.4	59.4	75.0	78.1	81.3	81.3

	Species	9	10	11	12	13	14	15
1	*HV punctigera*	.783	.706	.762	1.803	1.198	1.476	1.678
2	*HV armigera*	.959	.953	.973	1.974	.832	1.475	1.323
3	*HV assulta*	.661	.602	.612	1.938	.878	1.248	1.126
4	*HT punctifera*	.638	.513	.621	1.850	.940	1.243	1.159
5	*"H" rubrescens*	.795	.852	.915	1.469	1.149	1.507	1.386
6	*HH eodora*	.117	.045	.122	1.581	.997	1.404	2.055
7	*HH sp.1*	.161	.101	.091	1.589	1.059	1.363	2.661
8	*HH clathrata*	.389	.159	.223	1.444	1.139	1.059	2.326
9	*HH aberrans*	*****	.164	.197	1.446	1.038	1.454	2.170
10	*HH sp.2*	6.3	*****	.105	1.370	.956	1.361	2.170
11	*HH moribunda*	6.3	3.1	*****	1.333	1.012	1.420	2.416
12	*CA angustula*	74.2	67.7	71.0	*****	2.425	1.777	1.493
13	*SP litura*	58.1	54.8	61.3	90.0	*****	1.274	1.457
14	*AG infusa*	64.5	61.3	58.1	63.3	63.3	*****	1.223
15	MY convecta	81.3	75.0	78.1	71.0	64.5	64.5	*****

Unlike the other bands on these gels, this pink band was not visible when the PGK and ALD gels were counterstained. This pink band was probably due to a pigment from the moths, which also gave the moth homogenates from *C. angustula* a pinkish tinge.

Once *C. angustula, A. infusa* and *M. convecta* were eliminated from the analyses, several loci were also discarded, because for the ingroup taxa they only represented symplesiomorphies (derived character states shared by all taxa) and would not contribute any information on phylogenetic relationships. The loci discarded were *Gapd*, *Fh*, *αGpd*, *Pgk* and *Ald*. This left 27 loci and 12 species, including the outgroup *S. litura*, for the phylogenetic analyses.

From the allele frequency data (Appendix 1) two main groups of taxa were apparent. The *Helicoverpa*, *"Heliothis"* and *Heliothis* species formed one group, and differed from all the *Heliocheilus* species by fixed allele differences at the *Enol*, *Me* and *Mdh-1* loci , and by a mixture of fixed or frequency differences at the *Gpi*, *Pgm*, *Mpi*, *Ak-2*, *Got-2*, *Hex-2*, *Pep A-1*, *Pep B-1* and *Pep D* loci. These two groups had most alleles in common at the *Ak-1*, *Icd-1*, *Got-1*, *Hex-1*, *Pep A-2* and *Pep B-2* loci, with most of the variation occurring within the *Helicoverpa* /*"Heliothis"* /*Heliothis* group.

The Cavalli-Sforza and Edwards arc distances (Table 1.6) among *Heliocheilus* species ranged from 0.302 - 0.601. Among the *Helicoverpa* /*"Heliothis"*/*Heliothis* species group the range was 0.644 - 0.755. The arc distance between *Heliothis punctifera* and the *Helicoverpa* species ranged from 0.675 - 0.744.

The only published electrophoretic analysis of any of the taxa in this study is one comparing *Helicoverpa armigera* and *H. punctigera* (Daly and Gregg 1985). The Nei genetic distance between these species, in their study, was 0.330, based on 27 loci. This compares with a Nei distance of 0.474, based on 32 loci, in this current study (Table 1.5). When both data sets were reanalysed, using only the 21 loci that the studies had in common, the Nei distance values between *H. armigera* and *H. punctigera* were 0.440

Table 1.6. Matrix of Cavalli-Sforza arc distances for 12 taxa.
Abbreviations for genera: *HV: Helicoverpa, HT: Heliothis, "H": "Heliothis", HH: Heliocheilus, SP: Spodoptera.*

Species	2	3	4	5	6	7	8	9	10	11	12
1.*HV punctigera*	.666	.686	.744	.725	.762	.784	.813	.789	.762	.783	.869
2.*HV armigera*	****	.644	.706	.755	.837	.849	.880	.846	.854	.849	.782
3.*HV assulta*		****	.675	.715	.731	.786	.823	.773	.735	.736	.816
4.*HT punctifera*			****	.705	.695	.727	.742	.751	.672	.725	.813
5.*"H" rubrescens*				****	.806	.825	.814	.789	.808	.811	.864
6.*HH eodora*					****	.357	.452	.403	.302	.410	.834
7.*HH* sp.1						****	.465	.439	.385	.388	.851
8.*HH clathrata*							****	.601	.405	.487	.861
9.*HH aberrans*								****	.466	.464	.854
10.*HH* sp.2									****	.395	.826
11.*HH moribunda*										****	.849
12.*SP litura*											****

and 0.447 for the Daly and Gregg (1985) data and this study respectively, indicating very close agreement for given loci. This illustrates that the analysis of allozymes from different sets of loci can yield different estimates of genetic distance.

1.3.2 Phylogenetic analysis

The shortest tree produced by the distance Wagner analysis, calculated with Cavalli-Sforza and Edwards arc distances, is given in Fig. 1.3. In this tree the branch lengths are proportional to the distances. The *Heliocheilus* species form a monophyletic group with relatively short genetic distances within the group. The *Heliocheilus* and "*Heliothis*" species also form a monophyletic group, with *Heliothis punctifera* outside this group, but with a shorter genetic distance to it than to the *Heliocheilus* group. Within the *Heliocheilus* group, *H. aberrans* is the most genetically distant.

The tree produced by the Fitch-Margoliash method, also based on Cavalli-Sforza and Edwards arc distances (Fig. 1.4) had the lowest "percent standard deviation" of 212 trees examined by the program. Again, the branch lengths are proportional to distances. The topology is similar to that of the distance Wagner tree, except for some details of the internal relationships. The relationships within the *Heliocheilus* group are different, however *H. aberrans* is still placed as the most distant of these species. The relationships within the *Helicoverpa* and "*Heliothis*" species is also different, with "*H*". *rubrescens* placed outside the *Helicoverpa* species.

The tree produced by the maximum likelihood method (Fig. 1.5) is that which gave the best fit to the input data from 920 trees examined. With this method, rough confidence intervals are calculated for each branch length, and negative lower bounds indicate that a particular branch is not well resolved and alternative arrangements may be acceptable. Branch lengths are proportional to distances between taxa. Again, the topology is very similar to those of the previous two methods. The relationships within the *Heliocheilus* group are the same as those given by the distance Wagner method. The *Helicoverpa*/ "*Heliothis*"/ *Heliothis* species are monophyletic, with *Heliothis punctifera*

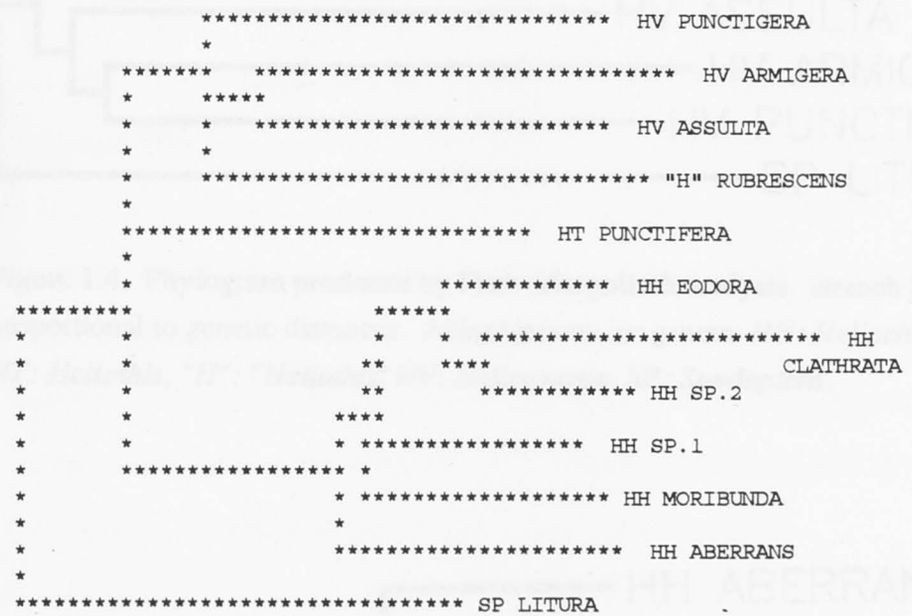

Figure 1.3. Phylogram produced by distance Wagner analysis. Branch lengths are proportional to genetic distances.
Abbreviations for genera: HV: *Helicoverpa*, "H":"*Heliothis*", HT: *Heliothis*, HH: *Heliocheilus*, SP: *Spodoptera*.

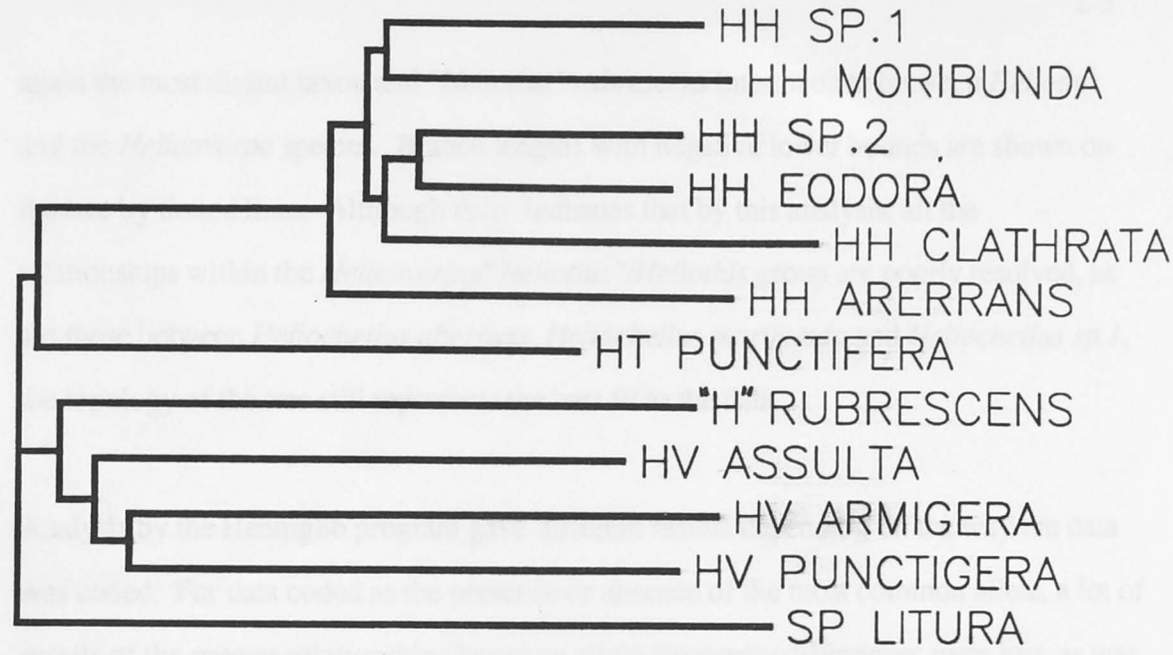

Figure 1.4. Phylogram produced by Fitch-Margoliash analysis. Branch lengths are proportional to genetic distances. Abbreviations for genera: *HH: Heliocheilus, HT: Heliothis, "H": "Heliothis", HV: Helicoverpa, SP: Spodoptera.*

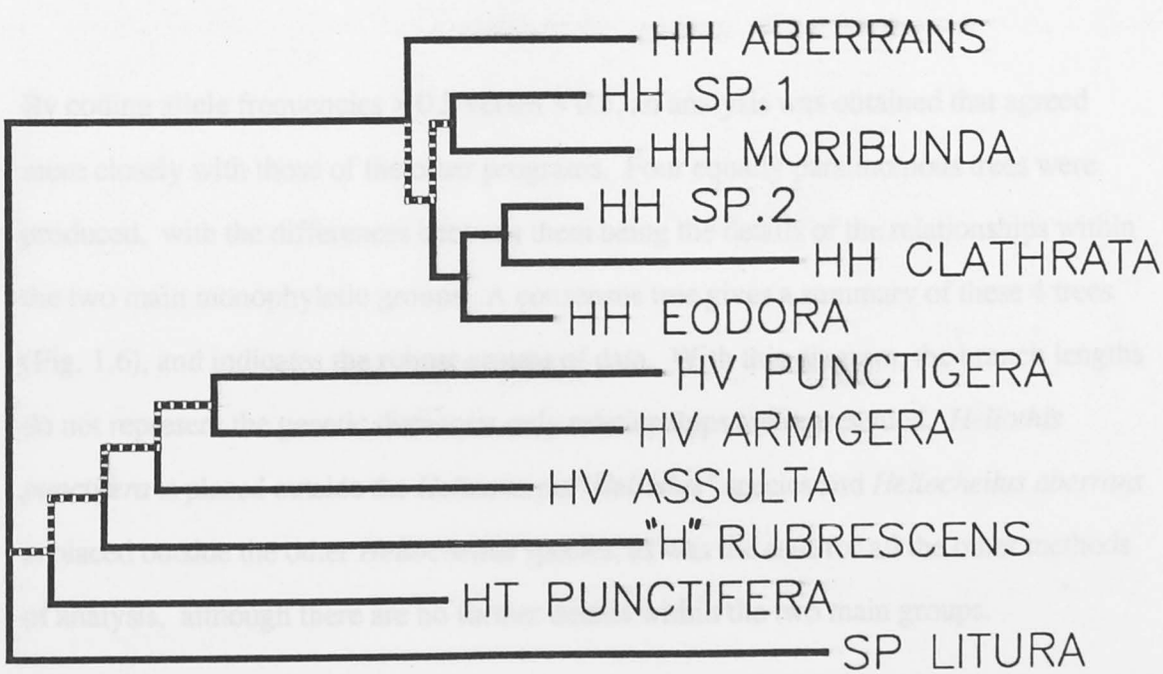

Figure 1.5. Phylogram produced by maximum likelihood analysis. Dotted lines indicate branch lengths with negative lower bounds. Branch lengths are proportional to genetic distances. Abbreviations for genera: *HH: Heliocheilus, HV: Helicoverpa, "H": "Heliothis", HT: Heliothis, SP: Spodoptera.*

again the most distant taxon and "*Heliothis*" *rubrescens* intermediate between *Heliothis* and the *Helicoverpa* species. Branch lengths with negative lower bounds are shown on the tree by dotted lines. Although this indicates that by this analysis, all the relationships within the *Helicoverpa*/"*Heliothis*"/*Heliothis* group are poorly resolved, as are those between *Heliocheilus aberrans*, *Heliocheilus moribunda* and *Heliocheilus sp.1*, the topology of the tree still represents the best fit to the data.

Analysis by the Hennig86 program gave different results depending on the way the data was coded. For data coded as the presence or absence of the most common allele, a lot of details of the species relationships based on allele frequency differences were lost, as was expected. The resultant tree, although still showing two distinct groups of taxa (the *Heliocheilus* and the *Helicoverpa*/"*Heliothis*"/*Heliothis* groups), showed internal relationships with no resemblance to those produced by the other programs. By this coding method, alleles at a frequency of 0.1 would be coded the same as those at a frequency of 1.0, giving undue weight to low frequency alleles.

By coding allele frequencies > 0.5 verses < 0.5, an analysis was obtained that agreed more closely with those of the other programs. Four equally parsimonious trees were produced, with the differences between them being the details of the relationships within the two main monophyletic groups. A consensus tree gives a summary of these 4 trees (Fig. 1.6), and indicates the robust groups of data. With this diagram, the branch lengths do not represent the genetic distances; only relationships are represented. *Heliothis punctifera* is placed outside the *Helicoverpa*/"*Heliothis*" species and *Heliocheilus aberrans* is placed outside the other *Heliocheilus* species, as was the case for all the other methods of analysis, although there are no further details within the two main groups.

1.4 Discussion

The proportion of polymorphic loci as observed by allozyme electrophoresis is on average 30% for invertebrates (Richardson et al. 1986), which is similar to the observed values for the moths in this study (a range of 19% to 44%). For *Helicoverpa armigera*

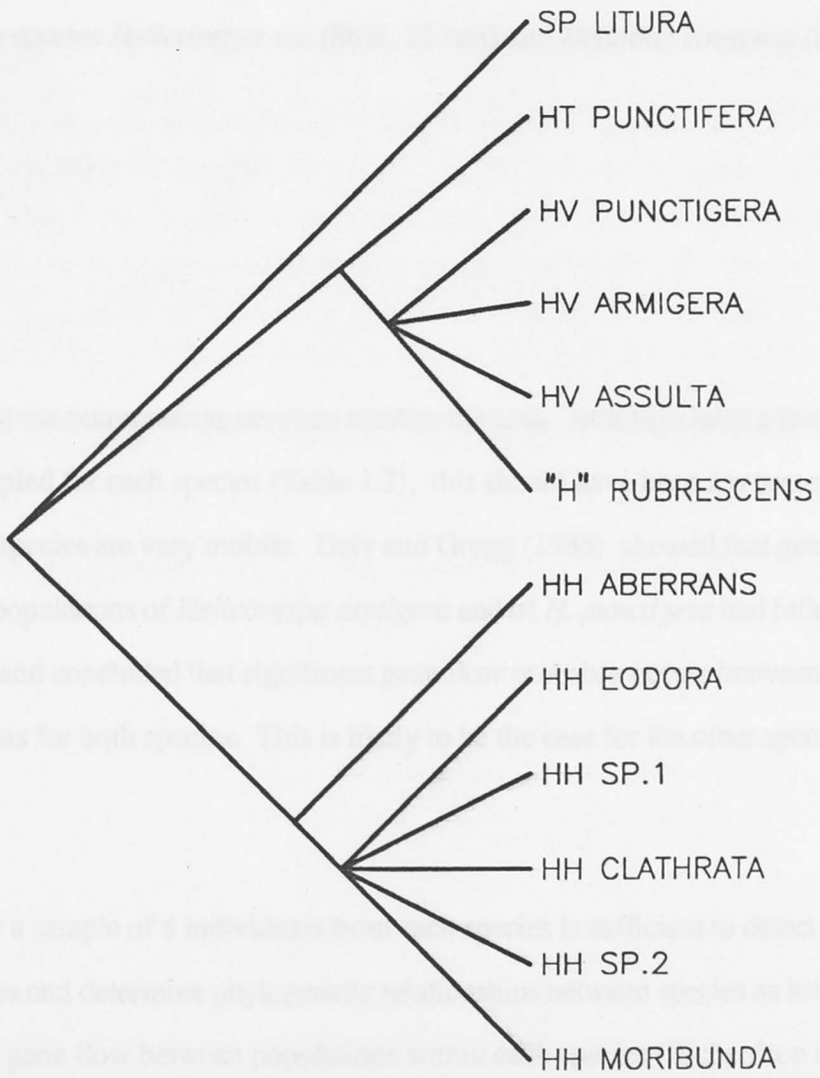

Figure 1.6. Consensus tree produced by Hennig86 analysis. Branch lengths do not reflect genetic distances.
Abbreviations for genera: *SP: Spodoptera, HT: Heliothis, HV: Helicoverpa, "H": "Heliothis", HH: Heliocheilus.*

* The data of Daly and Gregg (1985) and Sluss et al. (1978) given above, was of loci defined as polymorphic if the most frequent allele was at a frequency of less than 0.95 and 0.90 respectively. Although both these studies used up to 50 individuals per locus, there would be only a slightly greater chance of them detecting polymorphisms than was the case in the present study, with an average of 4 moths per locus, or 8 haploid genomes.

and *H. punctigera*, the measures were 25% and 41% respectively, at 32 loci, compared with 32% (28 loci) for both these species measured by Daly and Gregg (1985) in a study of several populations of each species. This contrasts with the very high variability in the American species *Helicoverpa zea* (86%, 22 loci) and *Heliothis virescens* (89%, 19 loci) (Sluss et al. 1978) although Daly and Gregg (1985) suggest that the American study may have been biased towards variable loci. *

Sample sizes were not large enough for meaningful measures of heterozygosity levels within species, nor were they intended to be, but were sufficiently large for phylogenetic analysis of the relationships between most of the taxa. Although only a few localities were sampled for each species (Table 1.2), this should have been a representative sample since the species are very mobile. Daly and Gregg (1985) showed that genetic distances between populations of *Helicoverpa armigera* and of *H. punctigera* had little geographic variation and concluded that significant gene flow probably occurs between widely spread populations for both species. This is likely to be the case for the other species in this study.

Generally a sample of 4 individuals from each species is sufficient to detect fixed differences and determine phylogenetic relationships between species as long as there is sufficient gene flow between populations within each species (Richardson et al. 1986). However this sample size is based on results of previous studies that showed it is quite rare for less than 10% of loci to show fixed differences between sibling species. For the *Heliocheilus* species in this study however, the fixed differences (Table 1.5) ranged from 0% (between *H. eodora* and *H. sp.2*) to less than 10% between all other species except between *H. aberrans* and *H. clathrata* (16%).

Measurements of dissimilarity are always underestimated when using allozyme data, because of cryptic variation within electromorphs. The *Heliocheilus* species in this study are all very similar electrophoretically, suggesting they have only recently diverged. It would be of interest to compare them by electrophoresis with non-Australian species of

* Each phylogenetic analysis method examines many trees, and produces one which represents the best fit between the input data and the given tree. This tree only represents the most likely relationships between species, based on the data available, and can not give a definitive taxonomy.

* Although the host plants of the Australian *Heliocheilus* spp. are unknown, other members of the same genus are restricted to grasses. From the specimens held in the Australian National Insect Collection in Canberra, it seems that these species were only occasionally collected, but from widespread localities of the drier interior of Australia. The limits of their distribution probably only reflect the limits of the collection sites. Two species, (*Heliocheilus sp.1* and *H sp.2*) were not previously represented in the collection. All these apparently closely related species share a similar habitat and probably only relatively recently evolved from a common ancestor.

the same genus.

*

All the methods of phylogenetic analysis gave similar results for the relationships between the genera. The Fitch-Margoliash and maximum likelihood methods suggest almost identical relationships between all the taxa and represent the most consistent phylogeny from the available data. The relationships among the *Helicoverpa* / "*Heliothis*" /*Heliothis* species by these analyses are in total agreement with that of Matthews (1987a). The *Heliocheilus* species are probably too similar electrophoretically for any certainty to be placed on their relationships within the genus, other than the placement of *H. aberrans* as more distant from the other species, as the Latin name "aberrans" suggests. The consistent placement of *Heliocheilus moribunda* with *H. sp.1* and of *H. clathrata* with *H. eodora* and *H. sp.2* by the distance Wagner, maximum likelihood and Fitch-Margoliash analyses suggests that these groupings are a true reflection of the given data. However larger sample sizes are probably needed where differences are only at the level of allele frequencies, and different arrangements might be found with a different data set.

*

Although the Hennig86 analysis produced an overall phylogeny in agreement with all the others, much important detail was lost by coding the data, and the well established relationships in the *Helicoverpa* /"*Heliothis*" /*Heliothis* group were not resolved.

The relative electrophoretic mobility of alleles should reflect their systematic affinity to some extent, although the probability of sequence changes leading to mobility convergence may be high. The large mobility differences between *Calophasidia. angustula* and the other taxa at a number of loci is further evidence that this species is indeed genetically quite distinct from the ingroup taxa and the other outgroup taxa. *C. angustula* (Stiriinae)was more different, electrophoretically, to the Heliothinae species than was *Spodoptera litura* (Amphipyrinae), *Mythimna convecta* (Hadeninae) or *Agrotis infusa* (Noctuinae). This disagrees with recent classifications which place the Stiriinae and the Heliothinae as sister groups. The level of divergence between *C. angustula* and the other species in this study was at the limits of usefulness of electrophoresis for

determining relationships. However, electrophoresis would certainly be useful for helping to resolve the relationships within the species currently classed as Stiriinae, world-wide.

1.5 Conclusion

Allozyme electrophoresis has proved to be a useful tool for taxonomic studies. In this study, the phylogenetic analysis of the electrophoretic data has produced a taxonomy in agreement with the most recent and thorough morphological analysis of the Heliothinae (Matthews 1987a). The *Helicoverpa* species form one group with *H. armigera* and *H. punctigera* the most closely related. "*Heliothis*" *rubrescens* is intermediate between *Helicoverpa* spp. and *Heliothis punctifera*. The *Heliocheilus* species are a distinct group within the Heliothinae, *H. aberrans* being more distant genetically than the other very closely related species. Further details of the degree of divergence between species have been added, showing the very close relationships among the Australian *Heliocheilus* species, and a much greater diversity among the *Helicoverpa*, "*Heliothis*" and *Heliothis* species.

Calophasidia angustula, classified as a member of the Stiriinae, does not seem to be as closely related to the Heliothinae as the other species used for outgroup analysis. The tentative placement of the Heliothinae and Stiriinae as sister groups, based on morphological and ecological characters, is not supported by the electrophoretic data. Alternatively, *C. angustula* may not be closely related to the American Stiriinae, which was the group linked with the Heliothinae.

Allozyme electrophoresis would be a useful first step for resolving relationships between taxa that have not been extensively studied morphologically. It is most useful at the species and genus level and of only limited use at the level of subfamilies within the family Noctuidae. It is certainly a useful alternative to the traditional morphological taxonomy methods, since it provides character sets independent of morphology.

Part 2: Cytogenetics of *Helicoverpa armigera* and *H. punctigera*.†

2.1 Introduction

2.1.1 General cytology of the Lepidoptera

The chromosomes of Lepidoptera are generally thought to be holocentric (White 1973), and are typically relatively small, making observations of details difficult. Other than by differences in their size, individual chromosome pairs can not usually be identified. Unlike most animals, the female is the heterogametic sex. The sex chromosomes are usually XX in males and XY in females, although there are some exceptions to this. The Lepidoptera have a greater range in chromosome number than any other animal group (from 7 to about 220 pairs), however there is a strongly marked mode at 29-31, with 31 the most common number of pairs (White 1973). The most common number of chromosomes reported in the Noctuidae is also 31 pairs (range 29-34) (Robinson 1971). Female meiosis is thought to be achiasmatic for all Lepidoptera, although this has only been confirmed for a limited number of species.

Only limited cytological information is available for the Heliothinae. Chen and Graves (1970) studied spermatogenesis in the North American pest *Heliothis virescens*, with a view to releasing sterile males as a control method. They reported 31 pairs of chromosomes in the male. This chromosome count was confirmed for male and female *H. virescens*, and also for *H. subflexa* in a study of mitotic cells by Roehrdanz (1990) who also gave evidence that the sex chromosomes were XX in males and XY in females.

This cytological study of *Helicoverpa armigera* and *H. punctigera*, the most economically damaging insect pests in Australian crops, aims to determine the karyotype of each species, identify any distinguishing features between chromosomes and between species, to evaluate the use of cytological characters as taxonomic characters for these species, and

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† Part of this work has been published in Genome, 32: 967-971, 1989, and has been attached to this thesis in appendix 2.

to determine whether female meiosis is achiasmatic. The latter would imply the absence of genetic crossing-over. The absence of crossing-over in female Lepidoptera greatly simplifies the construction of linkage maps, which would be useful for determining genetic linkage between genes conferring resistance to pesticides and biocontrol agents in these pest species.

2.1.2 Achiasmatic meiosis

Achiasmatic meiosis in one sex is a mechanism that appears to have arisen independently in a number of groups and the mechanism varies between groups. In insects it has been reported in Lepidoptera and Trichoptera, some Diptera, and in isolated genera of mantids, roaches and grasshoppers. It has also been observed in some scorpions, copepods and mites. It has not been observed at all in vertebrates, and in plants only in a few grasses (White 1973). In all these cases, the other sex is known to or thought to have normal chiasmatic meiosis, so recombination can still occur.

It is generally presumed that the absence of genetic crossing-over, inferred from genetic studies, is associated with the absence of chiasmata; the absence of the physical crossing of chromatids from different homologues in a bivalent, observed by cytological examination. This association has been well documented for the achiasmatic meiosis of male *Drosophila* spp. (White 1973). In Lepidoptera and *Drosophila* spp. it is the heterogametic sex which is achiasmatic.

Since achiasmatic female meiosis has been confirmed in only a small proportion of Lepidoptera, it is important to investigate this aspect for other species that are to be used for linkage studies. For example in *Drosophila*, one species *D. ananassae* has been shown to have recombination in males (Hinton 1970) and is the only exception that has been found in this genus.

Achiasmatic female meiosis has been observed, by cytological studies, in 12 families of Lepidoptera and in the closely related order Trichoptera (Table 2.1). Additionally,

Table 2.1. Reports of achiasmatic female meiosis from cytological studies in Lepidoptera and Trichoptera.

Family	Species	Reference
Lepidoptera		
Micropterigidae	*Micropteryx aureatella*	Suomalainen 1969b
Eriocraniidae	*Eriocrania semipurpurella*	Suomalainen 1969b
Hepialidae	*Hepialus hecta*	Suomalainen 1969b
Incurvariidae	*Incurvaria pectinea*	Suomalainen 1969b
Pyralidae	*Ectomylois ceratoniae*	Morag et al. 1983
	Ephestia kuehniella	Traut 1977
Geometridae	*Cideria* species	Suomalainen 1965
Pieridae	*Pieris napi*	Bigger 1975
Nymphalidae	*Charex jasius*	Trentini & Marini 1986
	Agraulis vanillae	Suomalainen et al. 1973
	Dryadula phaetusa	Suomalainen et al. 1973
	Heliconius (7 species)	Suomalainen et al. 1973
Bombycidae	*Bombyx mori*	Maeda 1939
	Bombyx mori & B.mandarina	Murakami & Imai 1974
Saturniidae	*Philosamia ricini*	Narang & Gupta 1979
	Antheraea compta & A.assamensis	Gupta & Narang 1981
Sphingidae	*Sphinx ligustri*	Nokkala 1987
Noctuidae	*Euxoa* (6 species)	Fontana 1976
Trichoptera	*Limnophilus decipiens & L.borealis*	Suomalainen 1966

limited cytological information suggests that female meiosis is probably achiasmatic in several species of Pyralidae and Tortricidae (Suomalainen 1969a,1971) and in *Erebia medusa* (Nymphalidae) (Federley 1938). Although primitive and advanced families are represented, this is only a small sample of the Lepidoptera, currently classified into 127 families (E. Nielsen and I. Common, pers. comm.). The only report of achiasmatic female meiosis from the large family Noctuidae is for species of *Euxoa* in the subfamily Noctuinae (Fontana 1976). There are no reports from the subfamily Heliothinae. Cytological and genetic studies have confirmed that the absence of chiasmata is accompanied by the absence of crossing-over in four Lepidoptera species; *Ephestia kuehniella* (Pyralidae) (Traut 1977), two species of *Heliconius* (Nymphalidae) (Turner and Sheppard 1975) and *Bombyx mori* (Bombycidae) (Sturtevant 1915, Turner 1979).

In addition, there have been no substantiated reports of chiasmatic female meiosis in the Lepidoptera. Suomalainen (1953) originally claimed that in females of the genus *Cidaria* (Geometridae), bivalents contained a single distal chiasma at metaphase I, but with improved techniques he subsequently discounted this and showed that female meiosis was in fact achiasmatic (Suomalainen 1965).

The mapping of chromosome markers is greatly simplified if there is no crossing-over of genetic material between homologous chromosomes in one sex during meiosis because only one marker is needed to define each chromosome as a linkage group. If two loci are tested by crossing a double heterozygous female with a double homozygous male, the two loci will show complete linkage if they are on the same chromosome, or complete independence if on different chromosomes. Linkage maps are currently being constructed for *H. virescens* using electrophoretic markers (D. Heckel, pers. comm.), however it has not been confirmed that female meiosis is achiasmatic in this species or any other Heliothinae.

2.1.3 Sex chromatin

Interphase somatic nuclei of most female Lepidoptera have a heteropycnotic body similar

to the sex chromatin of mammals (Smith 1945, Suomalainen 1969a, Ennis 1976). This heteropycnotic body, or heterochromatin, is a condensed and heavily staining part of the chromosomal material, and is referred to as sex chromatin when associated with the sex chromosomes. The heteropycnotic body in Lepidoptera is generally agreed to be sex chromatin associated with the Y chromosome (Ennis 1976); the presence of the body in the female only, indicates an XX male: XY female sex-determining mechanism, while its absence in both sexes indicates an XX male and X0 female. In four moth species with XX males and XYY females, two heteropycnotic bodies have been observed in the female somatic interphase nuclei (Suomalainen 1969a). Sex chromatin has been observed in female somatic cells of the North American species *Heliothis virescens* and *Heliothis subflexa*, and the observation of 62 chromosomes in somatic cells of both sexes was given as further evidence that these species have XY females and XX males (Roehrdanz 1990).

2.1.4 C-banding

C-bands are generally associated with centromeric heterochromatin in monocentric chromosomes, and have only been successfully induced in a few groups of insects, for example in the grasshopper genus *Caledia* (Shaw et al. 1976). C-banding of the holocentric Lepidoptera chromosomes has had varied results. Goodpasture (1976) induced C-banding in pachytene chromosomes of two species of Hesperiidae butterflies. Bedo (1984) used a variety of banding techniques but was unable to induce banding in metaphase chromosomes of a Gelechiidae moth, but did observe C-banding in prophase chromosomes. In addition to reports of C-bands, poorly resolved G-bands were shown by Bigger (1975) from two butterfly species, but were criticised by Bedo (1984) as being "not convincing".

2.1.5 Synaptonemal complex

The synaptonemal complex is a tripartite structure located between homologously paired chromosomes during pachytene in meiosis. Its formation seems to be a necessary prerequisite for crossing-over. In typical chiasmatic meiosis, including that of male

Lepidoptera (King and Akai 1971, Traut 1977, Holm and Rasmussen 1980), the synaptonemal complex is eliminated after crossing-over in pachytene and the chiasmata hold the homologues together until metaphase I.

Achiasmatic meiosis in female Lepidoptera has been most extensively studied in the silk moth *Bombyx mori* (Rasmussen 1976, Rasmussen 1977, Rasmussen and Holm 1980). Here the synaptonemal complex is modified and retained between the homologues until metaphase I, maintaining the co-orientation of the homologues and ensuring their regular disjunction in the absence of crossing-over. The presence of the synaptonemal complex in female meiosis of Lepidoptera has also been reported for several Pyralid species (Przelecka 1972, Traut 1977, Morag et al. 1983). This contrasts with the achiasmatic male meiosis of *Drosophila* spp. where no synaptonemal complex is formed, and the homologous chromosomes are held together by a mechanism similar to that in somatic cells of the Diptera (White 1973).

2.2 Materials and methods

2.2.1 Strains and rearing conditions

One *H. punctigera* strain and several *H. armigera* strains were examined. The *H. punctigera* strain and one *H. armigera* strain were derived from composite field collections in Queensland, maintained as laboratory cultures for 15 and 9 years respectively, by R. E. Teakle (pers. comm.) A variety of *H. armigera* strains were derived from composite field collections from the Emerald Irrigation Area, Queensland, and the Namoi Valley, New South Wales, during 1985 and 1986. The insects were reared at 25°C by the procedure of Teakle and Jensen (1985). Under these conditions, eggs hatch after 3 days, followed by 5 or 6 larval instars and then a pupal stage starting 15-17 days after egg hatch. The whole life cycle takes about 40 days.

2.2.2 Mitosis. Air-dried preparations

In Lepidoptera, metaphase is the stage where chromosomes are the most condensed, and hence easiest to observe with a light microscope. In earlier stages of mitosis, the large

number of chromosomes are so elongated and tangled that detailed observations are difficult. Eggs 1 to 1.5 days old are most suitable for mitotic metaphase preparations because cell division is most active. This stage of egg development can be identified by the pale ring of yellow pigment that develops on the egg membrane, and darkens to brown at 2 days after laying. Live embryos were dissected from eggs and incubated in 0.05% colchicine in insect saline (7.0 g NaCl, 0.2 g $CaCl_2$, 1000 ml H_2O) for 1 h at room temperature. This solution was replaced by two changes of fixative (3:1 ethanol: acetic acid) and left for 1-2 h. The drug colchicine inhibits spindle formation so that nuclei are unable to proceed to anaphase, and so nuclei held at metaphase will accumulate. Colchicine also causes the two chromatids of each chromosome to separate more widely (White 1973).

After fixation, embryos were transferred to a microscope slide in a drop of 60% acetic acid, macerated with a flat ended rod, and the drop moved around the slide until all the liquid had evaporated. Thoroughly air-dried slides were stained with 10% Giemsa in phosphate buffer (0.1% KH_2PO_4 and 0.082% Na_2HPO_4 in water, pH 6.8) for 20 min, rinsed with buffer, followed by water and then allowed to dry at 37°C. Slides were mounted with DePex. Chromosomes were photographed at a magnification of X 2000.

2.2.3 Male meiosis. Air-dried preparations

Spermatocytes at metaphase I were found in the testes of final instar larval males from about 13 days old, but were most abundant in 14 or 15 day old larvae (one or two days before pupation). Whole testes were dissected from live larvae under insect saline, and fixed in 3:1 ethanol: acetic acid for at least 1 h or stored in fixative at 4°C for up to 3 weeks. Air-dried slides were prepared and stained using the method described in section 2.2.2.

2.2.4 Female meiosis. (1) Air-dried and squash preparations

Exhaustive attempts were made to observe female meiosis in air-dried preparations of ovaries dissected from late stage pupae or newly emerged adult females, or from

individual ovules dissected from the ovaries. Due to the large amount of yolk in an egg, and the small size of the chromosomes, it was not possible to observe female meiosis by this method. Slides were also prepared by squashing lightly spread tissue under a coverslip with Lacto propionic orcein stain, but no female meiosis was observed by this method.

2.2.5 Female meiosis. (2) Sectioned preparations

Successful observations were made using sectioned material. During oogenesis in Lepidoptera, the chromosomes are suspended at metaphase I in the mature ovules until fertilisation. Whole mature ovaries were dissected under insect saline from adult females 2 days after emergence, and fixed for at least 1 h in 3:1 ethanol: acetic acid. The ovaries were then dehydrated in ethanol (30 min in each of 95% ethanol and 2 changes of 100% ethanol) and embedded in LR-white resin (London Resin Co.). Sections 1.5 µm thick were cut using a Reichert OM-2 ultramicrotome with a glass knife, and transferred onto pre-cleaned slides in a drop of water and dried on a warm hot-plate for at least 1 day. Slides were stained with 20% Giemsa in phosphate buffer for 15 min, taking great care not to wash off the sections, then dried and mounted in DePex. Sections were also prepared from fixed testes, by the method above, for a direct comparison of male and female meiosis.

Although sectioned preparations of material for light microscope observations of chromosomes have been made in the past, usually with paraffin wax as the embedding medium, this technique has rarely been used more recently since air-dried spreading techniques were developed. By embedding the tissue in a resin, commonly used for electron microscope examination, very thin sections could be obtained, giving good resolution with the light microscope, of the very small Lepidoptera chromosomes. This sectioning technique proved to be more reliable than air-dried or squash techniques for Lepidoptera oocytes.

2.2.6 Sex chromatin

Interphase somatic cells from air-dried preparations of embryos, testes and ovaries were examined for evidence of sex chromatin. The same methods were used as described in sections 2.2.2, 2.2.3 and 2.2.4, for observations of mitosis, male meiosis and female meiosis.

2.2.7 C-banding

Attempts were made to induce C-banding of meiotic metaphase chromosomes to see if individual pairs of chromosomes could be identified. The C-banding technique is similar to that described by Shaw et al. (1976). Air-dried slide preparations of testes were made as described in section 2.2.3, but not mounted. A variety of incubation times and staining times were tried. The best results were obtained using the following method. Slides were immersed in saturated $Ba(OH)_2$, diluted 1:1 with distilled water, for 15 min, dipped in 0.2 N HCl to remove excess $Ba(OH)_2$, rinsed in distilled water, then incubated in 2 x SSC (17.52 g NaCl, 8.80 g Na Citrate, 1000 ml H_2O, adjusted to pH 7.2) at 65°C for 90 min. Slides were rinsed then stained with 10% Giemsa in phosphate buffer for 30 min, and allowed to dry.

2.2.8 Synaptonemal complex

Preparations of synaptonemal complexes were made from spermatocytes of *H. armigera* larvae, by some of the techniques used by Rowell (1987). Spermatocytes at pachytene were found in 8 day old (late 4th instar) larval testes. Microscope slides were coated with gelatin by dipping washed slides in a solution of 0.1% gelatin and 0.01% chrome alum, and allowing them to dry vertically. Single whole testes were dissected and put in an eppendorf tube in 0.5 ml of insect saline. The testis was cut open then gently macerated to give a uniform cell suspension. A single drop of this suspension was put in the middle of a gelatin coated slide with 2 drops of 0.03% "Trix" lemon dishwashing liquid (R. and C. Products Pty. Ltd. Australia) to spread the chromosomes. Best results were obtained when the slide was left for 5 min before adding 6 drops of paraformaldehyde fixative. The slide was dried, supported over a hotplate, for about 2 h, soaked in double distilled

water for 1 min to remove excess fixative then stained for 10 min in 10% Giemsa in phosphate buffer and mounted in DePex.

2.3 Results

2.3.1 Mitosis

At least 20 clearly resolved mitotic cells at metaphase were examined from at least 10 embryos for both species. The karyotypes of *H. armigera* and *H. punctigera* were found to be similar. At mitotic metaphase there were 62 chromosomes in a graded series of sizes, between 0.5 and 1μm long, but individual pairs could not be differentiated (Fig. 2.1). Since the sex ratio in *Helicoverpa* spp. larvae is approximately 1:1, as observed in laboratory cultures, it is likely that mitosis was observed from both male and female embryos, but no dimorphism was observed.

2.3.2 Male meiosis

In the male larvae of these *Helicoverpa* spp. the testes are paired yellow kidney-shaped bodies located dorsally, either side of the midline in the fifth abdominal segment. They increase in size through the final larval instar, then move together and are fused into one paler central body just before pupation.

Air dried preparations from testes were used to confirm the haploid number of 31 pairs of chromosomes in both species. Counts were made from 60 spermatocytes at meiotic metaphase I from 16 male *H. armigera* (Fig. 2.2), and 110 spermatocytes from 9 male *H. punctigera* (Fig. 2.3).

At metaphase I in both species, air-dried preparations (Fig. 2.4) and sections (Fig. 2.5) showed the bivalents were orientated parallel to the spindle fibres. The bivalents were clearly chiasmate, showing a mixture of dumb-bell and cross shapes. Although the shape of the bivalents was sometimes difficult to determine, it was estimated from air-dried preparations that between 15 and 21 bivalents per cell from *H. punctigera* ($\bar{x}$=18.3, SD=1.1, n=20) were dumb-bell shaped, compared with only 8-12 per cell from

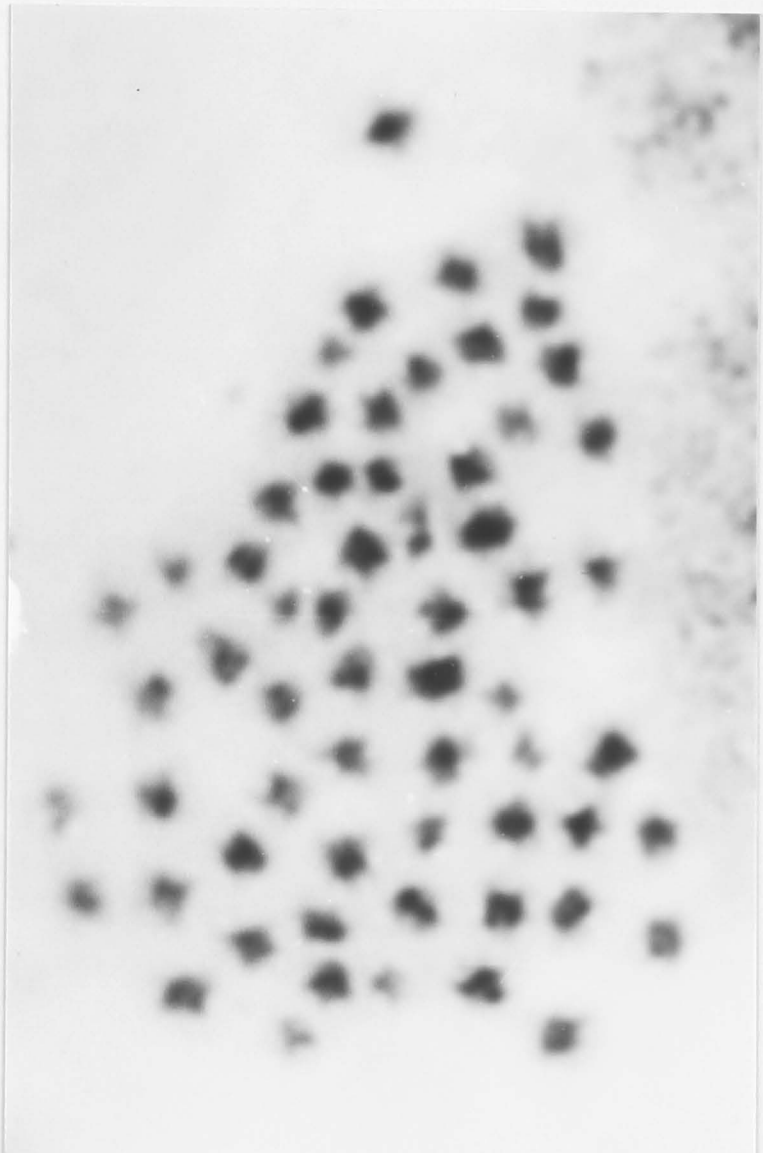


Figure 2.1. Mitotic metaphase in *H. armigera* with 62 chromosomes. Air-dried preparation.
Scale bar represents 2 µm.

Figure 2.2. Meiotic metaphase I in *H. armigera* spermatocyte with 31 bivalents. Air-dried preparation.
Scale bar represents 2 μm.

Figure 2.3. Meiotic metaphase I in *H. punctigera* spermatocyte with 31 bivalents. Air-dried preparation.
Scale bar represents 2 μm.

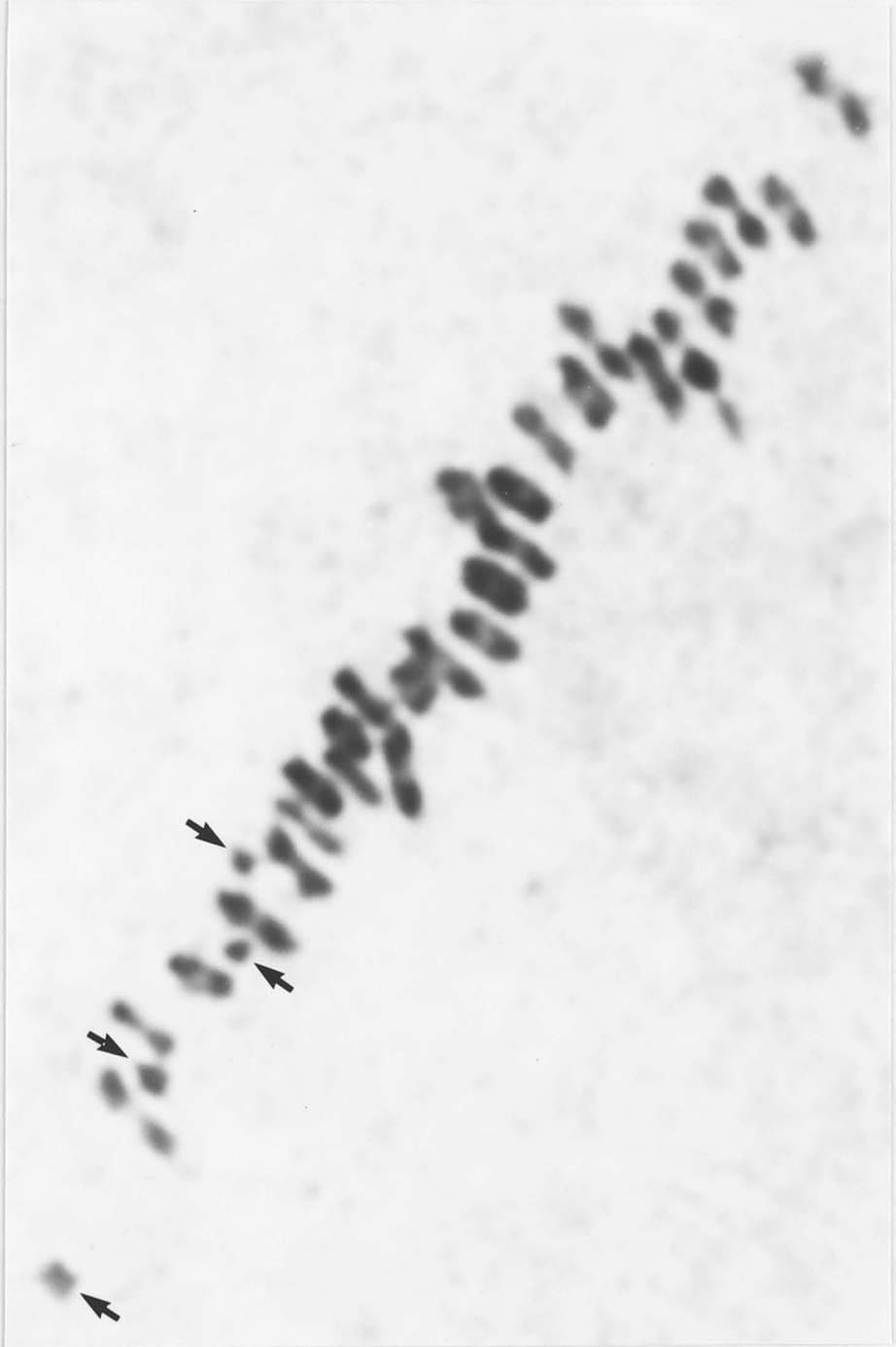


Figure 2.4. *H. punctigera* spermatocyte with 29 bivalents and 4 univalents (arrowed). Lateral view of metaphase plate. Air-dried preparation.
Scale bar represents 2 μm.

Figure 2.5. *H. punctigera,* sectioned preparation through testis showing two spermatocytes. The top spermatocyte shows a lateral view of the metaphase plate. The dumb-bell shaped bivalents have a single distal chiasma. The lower spermatocyte is sectioned parallel to the metaphase plate.
Scale bar represents 2 μm. ⌊⌋

H. armigera ($\bar{x}$=10.3, SD=1.8, n=8).

Some unusual spermatocytes were observed from *H. punctigera.* Eighteen percent of spermatocytes at first meiotic metaphase contained thirty bivalents and two univalents. The two univalents were always close to each other on the metaphase plate. In one spermatocyte, four univalents and 29 bivalents were observed (Fig. 2.4). The remaining 82% of nuclei had 31 bivalents. Spermatocytes with or without the univalents could be found within the same cyst. There was no evidence of these unusual spermatocytes from the variety of strains of *H. armigera* that were examined.

2.3.3 Female meiosis

Adult female Lepidoptera have paired ovaries, each comprising four strands of single lines of ovules, joined at the oviduct. Sections through the ovaries showed the nucleus located at the edge of each ovule close to the nutritive nurse cells of the ovary (Fig. 2.6), and close to the micropyle. The micropyle is the pore on the outer surface of the ovule, through which the sperm will enter.

Sections of oocytes at metaphase I showed the bivalents were aligned perpendicular to the spindle fibres, on the equatorial plate, with each pair of chromosomes lying parallel to each other, with a gap between, and no evidence of chiasmata (Fig. 2.7). Three female nuclei were observed from each species. Up to 7 bivalents were seen in each section, with at least 20 bivalents seen clearly from the series of sections from each nucleus.

2.3.4 Sex chromatin

Single sex chromatin bodies were seen in interphase somatic cells of female pupae and adults in preparations from whole ovaries from both *Helicoverpa* species. These somatic cells were the large polyploid nurse cells that are located in groups, alternating with the individual ovules in the ovary (Richards and Davies 1977). The highly polyploid nature of these cells allows them to synthesize and supply sufficient amounts of RNA to the large yolky oocytes (White 1973). In contrast to female somatic cells, interphase somatic

Figure 2.6. *H. punctigera* ovules. Sectioned preparation through ovary. Two ovules are shown. The oocyte (arrowed) can be seen at the edge of one ovule, close to the nurse cells.

Scale bar represents 20 μm. 

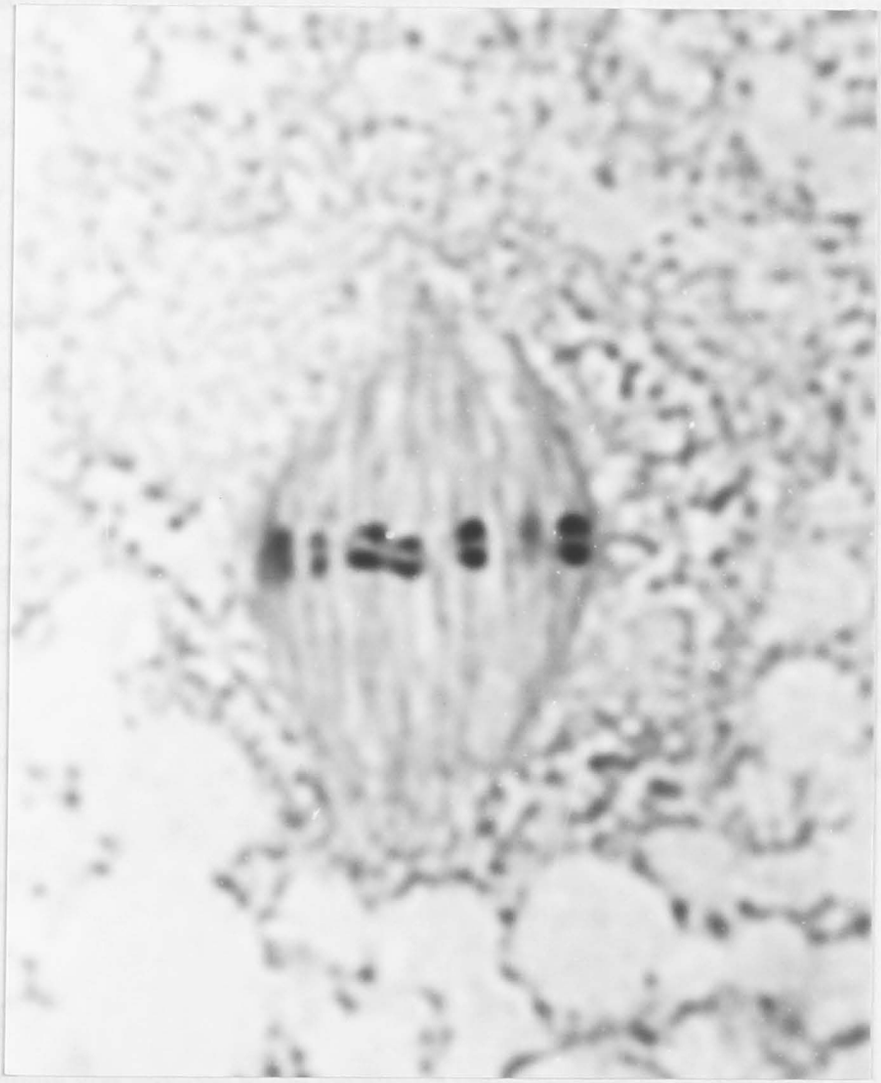


Figure 2.7. *H. punctigera* oocyte. Lateral view of metaphase plate. Sectioned preparation. The gap between the chromosomes of each bivalent shows that there are no chiasmata.

Scale bar represents 2 μm. ⌊——⌋

cells from testes preparations all had no sex chromatin bodies (Fig. 2.8).

The observation of sex chromatin in embryo preparations was less reliable; interphase cells were often difficult to identify. Sex chromatin was observed in 6 out of 16 embryo preparations, but the interphase cells were poorly resolved. Experience with rearing *Helicoverpa* species in the laboratory has shown their sex ratio is about 1:1 male to female, so the small proportion of female embryos identified by sex chromatin is probably due to the poor resolution of the interphase cells.

The sex chromatin in interphase cells from a 2 day old *H. armigera* embryo, presumed to be female, were particularly well resolved, and were distinctly heart shaped (Fig. 2.9). These interphase cells were most likely diploid, since the embryo cells were still undergoing normal mitotic divisions, as was evident by the large proportion of nuclei at other stages of mitosis. A single similarly shaped body was also observed in a few mitotic metaphase chromosomes from the same embryo preparation, and is likely to be the Y chromosome (Fig. 2.10). This heart shaped body was of similar size to most of the larger chromosomes. The sex chromatin body in the polyploid nurse cells was always rounded, and large relative to those seen in other somatic cells, and presumably represents several fused heterochromatic Y chromosomes.

2.3.5 C-banding

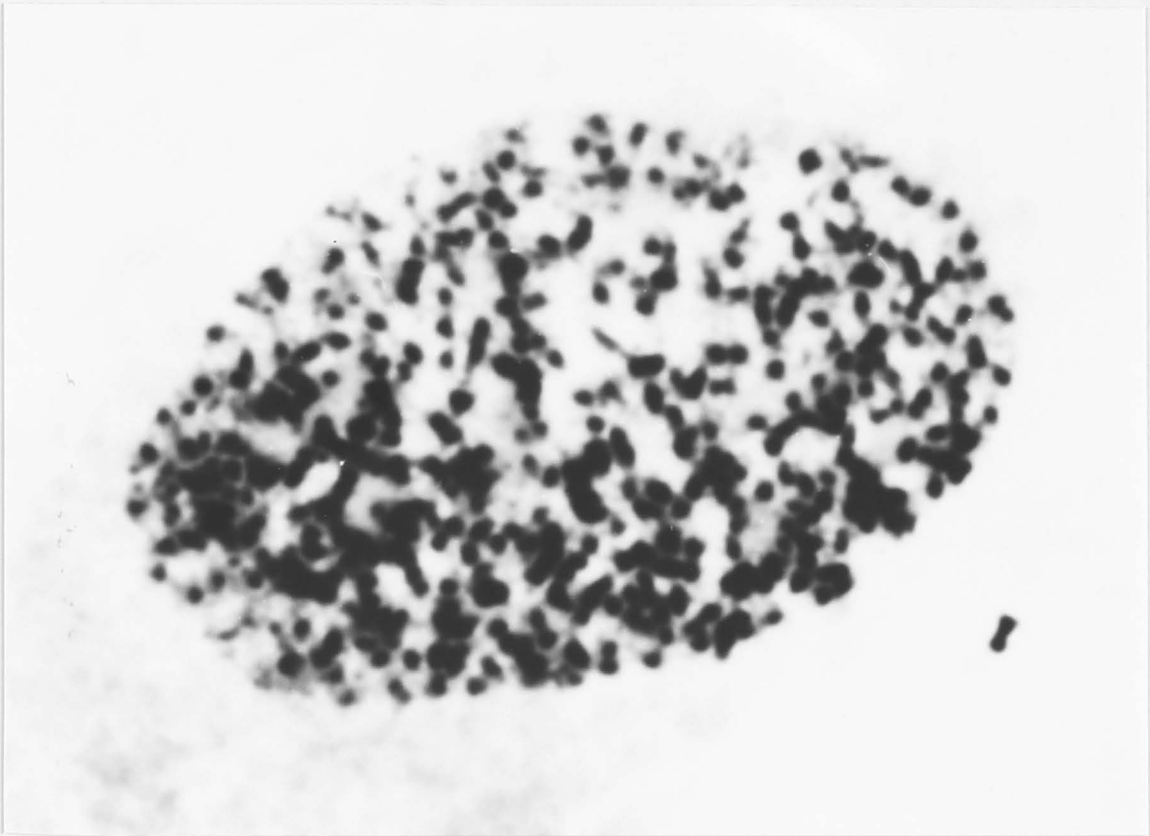
No C-banding was induced in the metaphase chromosomes of *H. armigera*, but bands were observed in early prophase chromosomes. These bands appeared as uniform banding on all chromosomes, and no distinctive sections could be resolved.

2.3.6 Synaptonemal complex

Synaptonemal complexes are best observed in prophase nuclei at pachytene, when they are least coiled, and before they are eliminated. Although synaptonemal complexes were observed, they were not spread sufficiently to identify individual chromosomes using the light microscope. This was expected, considering the large number of chromosomes

Figure 2.8. *H. punctigera* mitotic interphase nucleus from a male pupa. There is no sex chromatin. Air-dried preparation.
Scale bar represents 2 μm.

Figure 2.9. *H. armigera* mitotic interphase nucleus from a female embryo. The dark heart shaped body is the sex chromatin. Air-dried preparation.
Scale bar represents 2 μm.



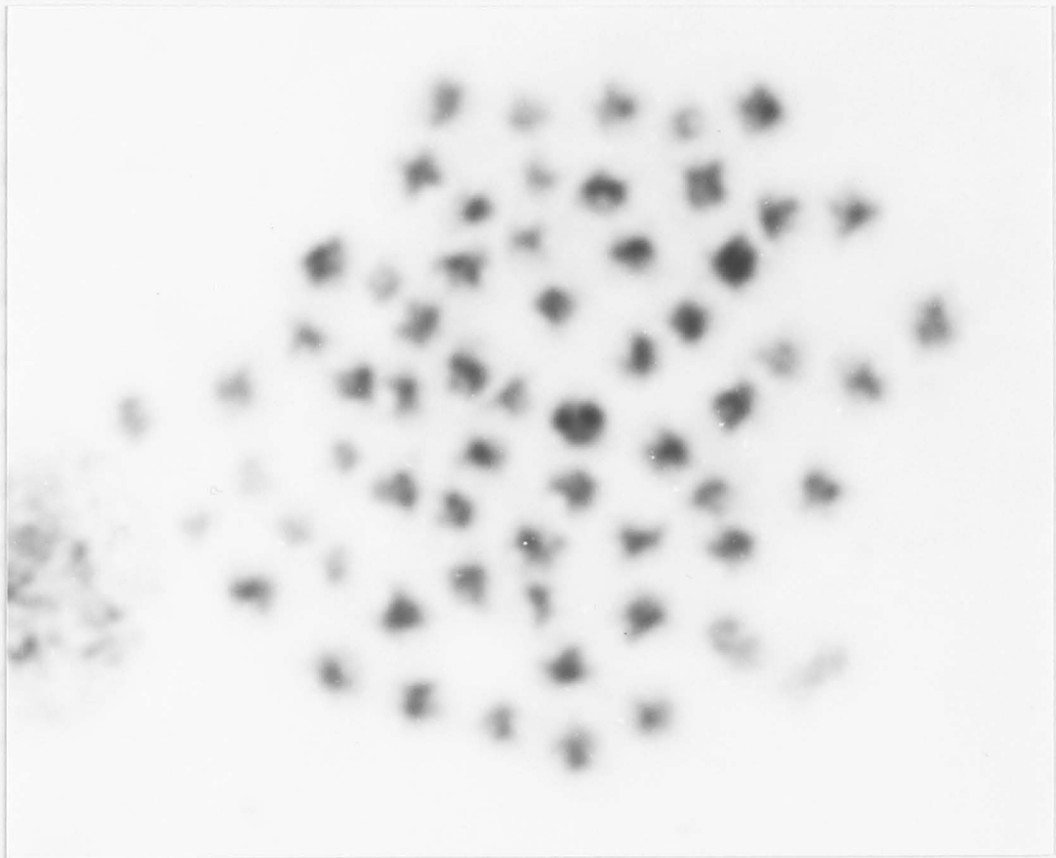


Figure 2.10. *H. armigera* mitotic metaphase nucleus from a female embryo. The heart shaped chromosome in the centre is probably the Y chromosome. Air-dried preparation. Scale bar represents 2 μm. ⌊——⌋

(31 pairs) in *H. armigera*, and the elongated nature of the pachytene chromosomes. No comparison was made with *H. punctigera* because it was considered that not enough detail would be observed to allow the two species to be differentiated.

2.4 Discussion

Helicoverpa armigera and *H. punctigera* have 31 pairs of chromosomes, which is the same number as reported in *Heliothis virescens* and *Heliothis subflexa* (Chen and Graves 1970, Roehrdanz 1990), and is the most common number for Noctuidae, and Lepidoptera as a whole (Robinson 1971).

The chromosomes in colchicine-arrested mitotic metaphase cells form a graded series of sizes, with no exceptionally large or distinctive chromosomes. Consequently, no individual pairs could be identified by size. This is also the case for the Noctuidae species *Heliocontia apicella* (Goodpasture 1976) and *Euxoa* spp. (Fontana 1976) and appears to be so in a photograph of mitotic chromosomes from a *Heliothis virescens*/*Heliothis subflexa* backcross (Roehrdanz 1990). A single larger pair of chromosomes has been reported in the Noctuidae species *Trichoplusi ni, Argyrogramma veruca* and *Prodenia ornithogalli* (Goodpasture 1976).

The metaphase I cells of males of the *Helicoverpa* species in this report clearly show a mixture of dumb-bell shaped bivalents and cross-shaped bivalents. A similar combination has also been demonstrated in other Lepidoptera by Goodpasture (1976) for species of Noctuidae and Hespiriidae, by Nokkala (1987) for Sphingidae and by Traut (1977) for Pyralidae. The photograph of metaphase I chromosomes from *Heliothis virescens* (Chen and Graves 1970) shows some dumb-bell shaped bivalents, but many bivalents are not well enough resolved to define their shape. The dumb-bell shapes represent extreme distal chiasmata. Whether the cross-shaped bivalents represent a single interstitial chiasma or two distal chiasmata at each end of the bivalent, could not be determined because earlier stages of meiosis were not observed.

The localisation of the chiasmata affects the overall level of genetic recombination, because the closer a chiasma is to the end of a chromosome, the less recombination will result. If the cross-shaped bivalents do indeed represent two distal chiasmata, then the level of recombination in such bivalents will not be greatly different to bivalents with a single distal chiasma, and the relative recombination frequencies for these *Helicoverpa* species will be similar. However, since spermatocytes at diakinesis were not observed it also could not be determined whether the chiasmata were originally interstitial, but had then "terminalised" by metaphase I. Although the occurrence of terminalisation of chiasmata in bivalents of monocentric chromosomes has been discounted, it has been well established for holocentric chromosomes, including those of some Lepidoptera (John 1990). Irrespective of the original localisation of the chiasmata, the number of bivalents at metaphase I with a single distal chiasma may be a consistent difference between these two species.

In some species of Lepidoptera, authors have reported that at metaphase I in males, all bivalents are dumb-bell shaped. These include some species of Saturniidae (Gupta and Narang 1981, Narang and Gupta 1979), Bombycidae (Murakami and Imai 1974) and Noctuidae (Fontana 1976).

The close proximity of the univalents seen in metaphase I in some *H. punctigera* spermatocytes (Fig. 2.4) suggests that they had recently been joined as bivalents, and are the result of precocious separation. Precocious separation of bivalents has been reported from some field-caught specimens of *H. armigera* from Emerald, Queensland in 1984 (C. Goodpasture, pers. comm.). Reports of this feature from other Lepidoptera are from *Hoplotarche lunana* (Noctuidae) and *Catopsilia phyranthe* (Pieridae) (Gupta 1964), and *Philosamia ricini* (Saturniidae) (Srivastava and Gupta 1962), where the presence of the univalents was attributed to either a lack of chiasma formation or a precocious separation of the homologues of the bivalents. Nuclei at the second meiotic division were not observed in *H. punctigera*. However counts from metaphase II plates from *H. lunana*, *C. phyranthe* and *P. ricini* were always of the normal haploid number, indicating that the

* Since there is no crossing-over in female meiosis, then construction of linkage maps would be practical. Any two genes found on the same chromosome would be selected together. For example if any genes in *Helicoverpa armigera* conferring resistance to pyrethroids were linked on the same chromosome to a gene conferring resistance to a bacterial endotoxin, then the management of both these chemical or biological control agents must take into account such linkage. Spraying *H. armigera* with pyrethroids could build up resistance to the bacterial endotoxin.

univalents were segregating alternatively and meiosis proceeded normally (Gupta 1964, Srivastava and Gupta 1962).

Electron micrographs of thin sections from oocytes of *Bombyx mori* (Rasmussen 1977) illustrate that the achiasmatic female meiosis in *B. mori* is similar to that observed in *H. armigera* and *H. punctigera* (Fig. 2.7), where the bivalents are aligned perpendicular to the spindle fibres, (equatorially) on the equatorial plate. In *B. mori* at metaphase I, homologous chromosomes were located on opposite sides of the equatorial plate separated by the modified synaptonemal complex. This mechanism for maintaining pairing until metaphase I probably also applies to *Helicoverpa* spp.

The orientation of the metaphase I bivalents from the male *Helicoverpa* spp. parallel to the spindle fibres (axially), contrasts with that in the female but seems to be typical for all the Lepidoptera reviewed in this study. The association of these orientations with the holocentric nature of the chromosomes or with chiasmatic or achiasmatic meiosis is not clear. In the Hemiptera, which have holocentric chromosomes, and chiasmatic meiosis in both sexes, bivalents are oriented axially in both sexes of some species, equatorially in both sexes of others or even axially in one sex and equatorially in the other (White 1973).

The achiasmatic female meiosis in *Helicoverpa* spp., demonstrated in this cytological study, is likely to be accompanied by the absence of crossing-over of genetic material, as has been well established for other insects (see section 2.1.2). *

The presence of sex chromatin in female somatic interphase nuclei but not in males is consistent with other Lepidoptera. The distinctive heart shape of the sex chromatin observed in some *H. armigera* nuclei could probably only be identified in a particular orientation, and could explain why it was seen in only a few nuclei. The corresponding heart shaped chromosome seen in some mitotic cells from embryos probably represents the Y chromosome, but is not a reliable feature for determining the sex because its recognition is dependant on its orientation.

The observation of C-banding in early prophase chromosomes but the failure to induce them in metaphase chromosomes is similar to the observations by Bedo (1984) of a Gelechiidae moth. With multiple bands seen uniformly on all chromosomes, and the 31 pairs of chromosomes all overlapping, individual chromosomes certainly cannot be identified from early prophase preparations.

2.5 Conclusion

The karyotypes of *H. armigera* and *H. punctigera* consist of 31 pairs of chromosomes of graded sizes with no pairs distinctive. The proportion of bivalents with distal chiasmata in spermatocytes at metaphase I of meiosis was greater for *H. punctigera* than *H. armigera*, but more populations need to be examined to confirm that this is a consistent difference. Precocious separation of bivalents was observed in some spermatocytes of *H. punctigera* at metaphase I. No useful identifying features of individual chromosome pairs, potentially useful as genetic markers, could be observed in chromosomes using several techniques. The small size and large number of chromosomes made light microscope observations of C-banded chromosomes and synaptonemal complex preparations of no use for identifying markers.

Chromosome number or form are unlikely to be useful taxonomic features for the Heliothinae. The lack of observable differences between *Helicoverpa armigera* and *H. punctigera,* which have the same number of chromosomes as *Heliothis virescens* and *H. subflexa*, suggests that other species of the *Helicoverpa*, *Heliothis* and the intermediate "*Heliothis*" genera are also likely to be similar. The difficulty of finding and identifying the larvae of other Heliothinae, including *Heliocheilus*, and of the Stiriinae, means that observations of larval spermatocytes, the most reliable cells in which to observe chromosomes, would not be possible.

The presence of sex chromatin in females only, along with chromosome counts of 62 in mitotic cells from both sexes, confirm that the sex chromosomes are XY in females and

XX in males. Although many variations in karyotype are found in Lepidoptera, the chromosomes of these *Helicoverpa* spp. are of the typical Noctuidae and Lepidoptera type, in size, shape and number.

The absence of chiasmata in female meiosis in *Helicoverpa* spp. is a feature shared with all of the limited number of other Lepidoptera previously examined for this feature. This physical observation most likely also represents the absence of crossing-over of genetic material and is an important prerequisite for genetic linkage studies aimed at understanding resistance to pesticides in these economically damaging pests.

3.1 Epilogue

Both parts of this thesis examine genetic variation between *H. armigera* and *H. punctigera*. The phylogenetic study is based on variation at the level of allozymes, and looks at these species in the context of the Australian Heliothinae. The cytological study examines genetic variation in the structure of the chromosomes, and how this may relate to the genome of both species.

The phylogenetic analysis of the Australian Heliothinae in this study, based on an analysis of allozyme data, is in agreement with the most recent and thorough morphological analysis. Allozyme electrophoresis is a relatively quick and reliable method and with appropriate sample sizes should be invaluable to resolve finer details of the relationships between species, particularly where this information is needed before biological control agents are released against the pest species.

Since the karyotype of both pest species *Helicoverpa armigera* and *H. punctigera* were so similar, no aspects of their karyotype would be useful taxonomic characters. However the demonstration that female meiosis is achiasmatic means that it is practical to construct genetic linkage maps, for the study of linkage between resistance to the variety of pesticides and biological control agents to which these species have been and will be exposed.

Appendix 1. Allele frequencies of all 15 species at 32 loci. (N) is the number of specimens analysed for each locus.

Key to species:

1 *Helicoverpa punctigera*
2 *Helicoverpa armigera*
3 *Helicoverpa assulta*
4 *Heliothis punctifera*
5 *"Heliothis" rubrescens*
6 *Heliocheilus eodora*
7 *Heliocheilus sp.1*
8 *Heliocheilus clathrata*
9 *Heliocheilus aberrans*
10 *Heliocheilus sp.2*
11 *Heliocheilus moribunda*
12 *Calophasidia angustula*
13 *Spodoptera litura*
14 *Agrotis infusa*
15 *Mythimna convecta*

	Species								
Locus	1	2	3	4	5	6	7	8	9
Gpi									
(N)	4	5	5	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.250	.125	.000
C	.125	.000	.100	.125	.000	.750	.625	.750	.625
D	.000	.000	.000	.000	.000	.125	.000	.125	.000
E	.875	1.000	.900	.875	.875	.125	.125	.000	.375
F	.000	.000	.000	.000	.125	.000	.000	.000	.000
G	.000	.000	.000	.000	.000	.000	.000	.000	.000
Pgm									
(N)	4	5	5	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.600	.000	.000	.000	.000	.000	.000
C	.125	.400	.200	.250	.000	.000	.000	.000	.000
D	.625	.500	.100	.375	.000	.000	.125	.375	.000
E	.250	.000	.100	.375	.000	1.000	.750	.625	.875
F	.000	.100	.000	.000	.875	.000	.125	.000	.125
G	.000	.000	.000	.000	.125	.000	.000	.000	.000
Mpi									
(N)	6	5	5	3	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.167	.000	.000	.125	.000	.000
C	.000	.400	.000	.833	.125	.625	.625	1.000	.500
D	.667	.400	.100	.000	.375	.375	.250	.000	.125
E	.333	.200	.700	.000	.500	.000	.000	.000	.250
F	.000	.000	.200	.000	.000	.000	.000	.000	.125
G	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.000	.000	.000	.000	.000
I	.000	.000	.000	.000	.000	.000	.000	.000	.000
Enol									
(N)	4	4	4	4	4	4	5	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.125	.000	1.000	1.000	1.000	1.000
C	1.000	1.000	1.000	.875	1.000	.000	.000	.000	.000
Me									
(N)	4	4	3	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.125	.000	.125
C	1.000	1.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	1.000	.875	1.000	.875
E	.000	.000	1.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	1.000	1.000	.000	.000	.000	.000
G	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.000	.000	.000	.000	.000

	Species								
Locus	1	2	3	4	5	6	7	8	9
Mdh-1									
(N)	4	4	3	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.250	.000	.000
B	.000	.000	.000	.000	.000	1.000	.750	1.000	1.000
C	.000	.000	.000	.125	.000	.000	.000	.000	.000
D	1.000	1.000	1.000	.875	1.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
Mdh-2									
(N)	4	4	4	4	4	4	4	4	4
A	.000	.500	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.250	.000	1.000	.000
D	.000	.500	1.000	1.000	.000	.750	1.000	.000	1.000
E	1.000	.000	.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	1.000	.000	.000	.000	.000
Ldh									
(N)	4	4	4	3	4	4	4	4	4
A	.000	1.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	1.000	.000	1.000	.000	.000	1.000	1.000	.000	1.000
D	.000	.000	.000	.000	1.000	.000	.000	1.000	.000
E	.000	.000	.000	1.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.000	.000	.000	.000	.000	.000	.000	.000	.000
Ak-1									
(N)	5	4	5	4	3	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.125	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.300	1.000	.900	1.000	1.000	1.000	1.000	.875	1.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.600	.000	.100	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.100	.000	.000	.000	.000	.000	.000	.000	.000
Ak-2									
(N)	4	4	4	4	5	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	1.000	.750	.000	.000	.000	.000	.000	.000
E	.000	.000	.250	.000	.000	1.000	1.000	1.000	1.000
F	1.000	.000	.000	1.000	1.000	.000	.000	.000	.000
Icd-1									
(N)	4	5	5	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.000	1.000	1.000	1.000	.000	1.000	.875	1.000	.500
D	1.000	.000	.000	.000	.000	.000	.125	.000	.500
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	1.000	.000	.000	.000	.000
Icd-2									
(N)	4	5	5	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.125	.000
C	.000	.000	.300	.000	.000	.000	.000	.500	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.600	1.000	.000	1.000	1.000	.375	1.000
F	.875	.900	.000	.000	.000	.000	.000	.000	.000
G	.125	.000	.100	.000	1.000	.000	.000	.000	.000
H	.000	.100	.000	.000	.000	.000	.000	.000	.000

	Species								
Locus	1	2	3	4	5	6	7	8	9
Gapd									
(N)	4	5	5	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.625	.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.375	1.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
Got-1									
(N)	5	4	4	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000	.125	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	1.000	1.000	1.000	1.000	1.000	1.000	.875	1.000	1.000
Got-2									
(N)	5	4	3	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	1.000	.125	.875	.250
B	.000	.000	.000	.000	.000	.000	.000	.125	.000
C	.000	.000	.000	.000	.000	.000	.875	.000	.125
D	.000	.000	.000	.000	.000	.000	.000	.000	.625
E	.000	.000	.000	1.000	.000	.000	.000	.000	.000
F	.000	1.000	1.000	.000	1.000	.000	.000	.000	.000
G	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	1.000	.000	.000	.000	.000	.000	.000	.000	.000
Pgd									
(N)	4	3	4	3	3	3	3	3	3
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.167	.167	.000	.000	.333
C	.000	.667	.000	1.000	.833	.833	1.000	1.000	.667
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	1.000	.333	1.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
Fh									
(N)	5	4	4	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
αGpd									
(N)	4	4	4	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Hex-1									
(N)	5	4	4	3	4	4	3	4	4
A	.000	.000	.000	.667	.000	.000	.000	.000	.000
B	.200	.000	1.000	.333	1.000	1.000	1.000	.500	1.000
C	.800	.000	.000	.000	.000	.000	.000	.500	.000
D	.000	1.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.000	.000	.000	.000	.000	.000	.000	.000	.000
Hex-2									
(N)	5	4	4	4	4	4	4	4	4
A	.000	.000	.000	.125	.000	.000	.000	.000	.000
B	.900	1.000	.000	.000	.125	.000	.000	.000	.000
C	.000	.000	.000	.000	.875	.000	.000	.000	.000
D	.100	.000	1.000	.875	.000	.125	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.125	.250
F	.000	.000	.000	.000	.000	.875	1.000	.000	.750
G	.000	.000	.000	.000	.000	.000	.000	.875	.000

	Species								
Locus	1	2	3	4	5	6	7	8	9
Pgk									
(N)	5	4	5	4	4	4	4	2	4
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
Pep.A-1									
(N)	4	4	4	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	1.000	.250	.000	1.000
D	.000	.000	.000	.250	.000	.000	.750	1.000	.000
E	.000	1.000	.000	.750	.000	.000	.000	.000	.000
F	.750	.000	.875	.000	1.000	.000	.000	.000	.000
G	.250	.000	.125	.000	.000	.000	.000	.000	.000
Pep.A-2									
(N)	4	4	3	4	3	4	4	4	4
A	.000	1.000	.667	.000	.000	.000	.000	.000	.000
B	1.000	.000	.333	1.000	.333	1.000	1.000	1.000	.000
C	.000	.000	.000	.000	.667	.000	.000	.000	1.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
Pep.B-1									
(N)	4	4	4	4	4	4	4	4	4
A	.000	.000	.375	.000	.000	.000	.000	.000	.000
B	.000	.000	.125	.000	.000	.000	.000	.000	.000
C	.875	1.000	.000	.875	.000	.000	.000	.000	.000
D	.000	.000	.500	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.750	.000	.000	.000	.125
F	.000	.000	.000	.000	.000	.500	.500	.500	.250
G	.125	.000	.000	.125	.250	.500	.500	.500	.500
H	.000	.000	.000	.000	.000	.000	.000	.000	.125
Pep.B-2									
(N)	4	4	4	4	3	4	4	4	4
A	.000	.125	.000	.000	.000	.000	.000	.000	.000
B	.000	.250	.125	.250	.000	.000	.000	.000	.000
C	.250	.000	.000	.250	.500	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
E	.750	.625	.875	.500	.167	1.000	1.000	1.000	.000
F	.000	.000	.000	.000	.333	.000	.000	.000	.000
G	.000	.000	.000	.000	.000	.000	.000	.000	1.000
Pep.D									
(N)	4	4	4	4	3	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.250	.375	.000	.000	.000	.000	.000	.000	.000
C	.500	.625	.375	.000	.167	.000	.000	.000	.000
D	.250	.000	.375	.000	.000	.000	.000	.000	.000
E	.000	.000	.125	1.000	.833	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.250	.375	.000	.625
G	.000	.000	.125	.000	.000	.625	.375	1.000	.000
H	.000	.000	.000	.000	.000	.125	.000	.000	.250
I	.000	.000	.000	.000	.000	.000	.250	.000	.000
J	.000	.000	.000	.000	.000	.000	.000	.000	.125
Ald									
(N)	4	3	4	3	3	3	3	3	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Acon-1									
(N)	4	5	5	4	4	4	4	4	4
A	.000	1.000	1.000	1.000	.625	.000	.000	.000	.000
B	1.000	.000	.000	.000	.375	1.000	1.000	1.000	1.000

	Species								
Locus	1	2	3	4	5	6	7	8	9
Acon-2									
(N)	3	4	5	4	3	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.375	1.000	1.000	.250
C	1.000	1.000	1.000	1.000	.000	.625	.000	.000	.750
D	.000	.000	.000	.000	1.000	.000	.000	.000	.000
Ao									
(N)	4	4	5	4	3	4	4	4	4
A	.250	.250	.000	.000	.000	.000	1.000	.250	.500
B	.375	.000	.800	1.000	.500	.500	.000	.750	.500
C	.375	.750	.200	.000	.500	.500	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000	.000
Hbdh									
(N)	5	4	4	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000	.125	.000	.000
B	.000	.000	.250	.000	.375	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.100	.000	.000	1.000	.625	1.000	.875	1.000	1.000
E	.000	.000	.750	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.900	1.000	.000	.000	.000	.000	.000	.000	.000
I	.000	.000	.000	.000	.000	.000	.000	.000	.000
G6pd									
(N)	3	3	4	4	4	4	4	4	3
A	.000	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000	.000	.000	.000
C	1.000	.000	.000	.000	.125	1.000	1.000	1.000	.833
D	.000	.000	.000	1.000	.000	.000	.000	.000	.000
E	.000	1.000	.375	.000	.875	.000	.000	.000	.167
F	.000	.000	.000	.000	.000	.000	.000	.000	.000
G	.000	.000	.625	.000	.000	.000	.000	.000	.000

	Species					
Locus	10	11	12	13	14	15
Gpi						
(N)	4	4	5	4	4	4
A	.000	.125	.000	.000	.000	.000
B	.125	.125	.000	.125	.125	.000
C	.875	.625	.000	.875	.750	1.000
D	.000	.000	.400	.000	.125	.000
E	.000	.125	.000	.000	.000	.000
F	.000	.000	.200	.000	.000	.000
G	.000	.000	.400	.000	.000	.000
Pgm						
(N)	4	4	5	4	4	4
A	.000	.000	.000	.000	.125	.000
B	.000	.000	.000	.000	.000	.000
C	.000	.125	.000	.000	.375	.875
D	.125	.250	.000	1.000	.500	.125
E	.875	.500	.200	.000	.000	.000
F	.000	.125	.800	.000	.000	.000
G	.000	.000	.000	.000	.000	.000

	Species					
Locus	10	11	12	13	14	15
Mpi						
(N)	4	4	5	4	4	4
A	.000	.000	.000	.000	.000	.875
B	.125	.000	.000	.125	.000	.125
C	.250	.500	.000	.750	.750	.000
D	.250	.250	.000	.125	.250	.000
E	.250	.125	.000	.000	.000	.000
F	.125	.125	.000	.000	.000	.000
G	.000	.000	.200	.000	.000	.000
H	.000	.000	.300	.000	.000	.000
I	.000	.000	.500	.000	.000	.000
Enol						
(N)	4	4	5	4	4	4
A	.000	.250	.000	.000	.000	.000
B	1.000	.750	.000	.875	.000	.000
C	.000	.000	1.000	.125	1.000	1.000
Me						
(N)	4	4	5	4	4	4
A	.000	.000	1.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	1.000	.000	.000
D	1.000	1.000	.000	.000	.000	.000
E	.000	.000	.000	.000	1.000	.000
F	.000	.000	.000	.000	.000	.000
G	.000	.000	.000	.000	.000	.500
H	.000	.000	.000	.000	.000	.500
Mdh-1						
(N)	4	4	5	4	3	4
A	.000	.000	.000	.000	.000	.000
B	1.000	1.000	1.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	1.000
E	.000	.000	.000	1.000	1.000	.000
Mdh-2						
(N)	4	4	5	4	4	4
A	.000	.000	.000	.000	.000	.000
B	.000	.000	1.000	.000	.000	.000
C	.250	.000	.000	.000	1.000	.000
D	.750	1.000	.000	1.000	.000	.000
E	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	1.000
Ldh						
(N)	3	4	4	4	4	3
A	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	1.000	.250	.000
C	1.000	1.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.750	1.000
F	.000	.000	.500	.000	.000	.000
G	.000	.000	.500	.000	.000	.000
Ak-1						
(N)	4	4	3	4	4	4
A	.125	.000	.000	.000	.000	.000
B	.000	.000	1.000	.000	.250	.875
C	.875	1.000	.000	1.000	.000	.000
D	.000	.000	.000	.000	.625	.125
E	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.125	.000
G	.000	.000	.000	.000	.000	.000

	Species					
Locus	10	11	12	13	14	15
Ak-2						
(N)	4	4	4	4	4	3
A	.000	.000	1.000	.000	.000	.000
B	.000	.000	.000	1.000	.000	.000
C	.000	.000	.000	.000	.000	1.000
D	.000	.500	.000	.000	.000	.000
E	1.000	.500	.000	.000	1.000	.000
F	.000	.000	.000	.000	.000	.000
Icd-1						
(N)	4	4	5	4	4	4
A	.000	.000	.000	.000	.000	.500
B	.000	.000	.000	1.000	.000	.500
C	1.000	1.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.625	.000
E	.000	.000	1.000	.000	.375	.000
F	.000	.000	.000	.000	.000	.000
Icd-2						
(N)	4	4	5	4	4	4
A	.000	.000	.000	1.000	.000	.000
B	.250	.000	1.000	.000	.000	.000
C	.000	.125	.000	.000	1.000	.000
D	.000	.000	.000	.000	.000	1.000
E	.625	.875	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	.000
G	.125	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.000	.000
Gapd						
(N)	4	4	5	4	4	4
A	.000	.000	.000	.000	.000	.000
B	1.000	1.000	.000	1.000	.000	.000
C	.000	.000	1.000	.000	.000	.000
D	.000	.000	.000	.000	.000	1.000
E	.000	.000	.000	.000	1.000	.000
Got-1						
(N)	4	4	5	4	4	4
A	.000	.000	.000	.000	.000	1.000
B	.000	.000	.000	.000	.375	.000
C	.000	.000	.000	1.000	.625	.000
D	.125	.500	1.000	.000	.000	.000
E	.875	.500	.000	.000	.000	.000
Got-2						
(N)	4	4	5	0	4	4
A	.500	.000	.000	.000	.000	.000
B	.000	.250	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000
D	.000	.500	.000	.000	.000	.000
E	.500	.250	.000	.000	.250	.000
F	.000	.000	.200	.000	.500	1.000
G	.000	.000	.800	.000	.000	.000
H	.000	.000	.000	.000	.250	.000
Pgd						
(N)	3	2	4	3	3	3
A	.000	.000	1.000	.000	.000	.000
B	.000	1.000	.000	.000	.000	.000
C	1.000	.000	.000	.000	1.000	.000
D	.000	.000	.000	1.000	.000	.000
E	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.000	1.000

	Species					
Locus	10	11	12	13	14	15
Fh						
(N)	4	4	5	4	4	4
A	.000	.000	.000	.000	1.000	.000
B	.000	.000	1.000	.000	.000	1.000
C	.000	.000	.000	1.000	.000	.000
D	1.000	1.000	.000	.000	.000	.000
αGpd						
(N)	4	4	5	4	4	4
A	.000	.000	.000	.000	1.000	.000
B	.000	.000	1.000	.000	.000	1.000
C	1.000	1.000	.000	1.000	.000	.000
Hex-1						
(N)	4	4	5	3	4	4
A	.000	.000	.000	.000	.000	.000
B	1.000	1.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.750
D	.000	.000	.000	.167	.125	.000
E	.000	.000	.000	.167	.000	.000
F	.000	.000	.000	.666	.500	.125
G	.000	.000	1.000	.000	.375	.125
Hex-2						
(N)	4	4	4	3	4	3
A	.000	.000	.750	.000	.000	.000
B	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	1.000	.000	.000
D	.000	.000	.250	.000	.000	1.000
E	.000	.500	.000	.000	1.000	.000
F	.750	.500	.000	.000	.000	.000
G	.250	.000	.000	.000	.000	.000
Pgk						
(N)	4	4	0	4	4	4
A	1.000	1.000	.000	1.000	.000	1.000
B	.000	.000	.000	.000	1.000	.000
Pep.A-1						
(N)	4	4	6	4	4	4
A	.000	.000	.000	1.000	.000	.000
B	.000	.000	.000	.000	.000	.250
C	.250	.250	.000	.000	.000	.750
D	.750	.750	.500	.000	.000	.000
E	.000	.000	.500	.000	.125	.000
F	.000	.000	.000	.000	.625	.000
G	.000	.000	.000	.000	.250	.000
Pep.A-2						
(N)	4	4	2	4	0	4
A	.000	.000	.000	1.000	.000	.875
B	1.000	1.000	.000	.000	.000	.000
C	.000	.000	1.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.125
Pep.B-1						
(N)	4	4	5	4	4	4
A	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	1.000	1.000
E	.000	.125	.000	.000	.000	.000
F	.000	.250	.000	.000	.000	.000
G	1.000	.625	1.000	.000	.000	.000
H	.000	.000	.000	1.000	.000	.000

Locus	Species 10	11	12	13	14	15
Pep.B-2						
(N)	4	4	4	4	4	4
A	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.625	.000
C	.000	.000	.625	.000	.125	.000
D	.000	.000	.000	.000	.000	1.000
E	1.000	1.000	.000	1.000	.250	.000
F	.000	.000	.375	.000	.000	.000
G	.000	.000	.000	.000	.000	.000
Pep.D						
(N)	4	4	6	4	4	4
A	.000	.000	.000	1.000	.000	.000
B	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.375	.250
D	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.500	.625
F	.000	.125	.167	.000	.125	.000
G	.750	.875	.833	.000	.000	.125
H	.000	.000	.000	.000	.000	.000
I	.000	.000	.000	.000	.000	.000
J	.250	.000	.000	.000	.000	.000
Ald						
(N)	3	2	2	3	4	4
A	.000	.000	.000	.000	.000	1.000
B	1.000	1.000	1.000	1.000	1.000	.000
Acon-1						
(N)	4	4	5	4	4	4
A	.000	.000	.000	1.000	.000	1.000
B	1.000	1.000	1.000	.000	1.000	.000
Acon-2						
(N)	5	4	5	3	4	4
A	.000	.000	1.000	.000	.000	.500
B	.200	1.000	.000	.000	1.000	.000
C	.800	.000	.000	1.000	.000	.500
D	.000	.000	.000	.000	.000	.000
Ao						
(N)	4	4	5	4	4	4
A	.000	.500	.000	.000	.000	.000
B	1.000	.500	.000	.750	.750	.500
C	.000	.000	.000	.250	.250	.500
D	.000	.000	1.000	.000	.000	.000
Hbdh						
(N)	3	4	6	4	4	4
A	.000	.000	.000	.000	.000	.000
B	.000	.125	.750	.000	.000	.000
C	.000	.000	.000	.000	.125	.000
D	1.000	.875	.250	.625	.000	.000
E	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.875	.000
G	.000	.000	.000	.375	.000	.000
H	.000	.000	.000	.000	.000	.000
I	.000	.000	.000	.000	.000	1.000
G6pd						
(N)	3	3	4	4	2	4
A	.000	.000	.250	.000	.000	.000
B	.000	.000	.750	.000	.000	.000
C	1.000	1.000	.000	.000	.000	.000
D	.000	.000	.000	.000	1.000	1.000
E	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	1.000	.000	.000
G	.000	.000	.000	.000	.000	.000

Appendix 2.

Karyotype and achiasmatic female meiosis in *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae)

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The economically important pest species *Helicoverpa armigera* and *H. punctigera* have a karyotype consisting of 31 pairs of chromosomes. The chromosomes are in a graded series of sizes such that pairs cannot be differentiated. Cytological evidence suggests that female meiosis is achiasmatic. Precocious separation of bivalents into univalents at metaphase I was observed in some spermatocytes of *H. punctigera*. This species also had a consistently greater number of bivalents with fully terminalised chiasmata in each spermatocyte at male metaphase I than *H. armigera*.

Key words: Lepidoptera, *Helicoverpa*, achiasmatic meiosis, karyotype.

FISK, J. H. 1989. Karyotype and achiasmatic female meiosis in *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae). Genome, **32** : 967–971.

Des espèces d'insectes nuisibles d'importance économique, le *Helicoverpa armigera* et le *H. punctigera*, ont un caryotype constitué de 31 paires de chromosomes. Ces chromosomes sont assortis en une série de dimensions telles que les paires ne peuvent être distinguées. Des évidences cytologiques suggèrent que la méiose femelle est achiasmatique. Une séparation hâtive des bivalents en univalents a été observée à la métaphase I chez certains spermatocytes de *H. punctigera*. Cette espèce, par comparaison avec le *H. armigera*, s'est aussi avéré posséder un plus grand nombre de bivalents à la métaphase I, présentant des chiasmas terminaux dans chaque spermatocyte mâle, et ce, de façon constante.

Mots clés : Lépidoptères, *Helicoverpa*, méiose achiasmatique, caryotype.

[Traduit par la revue]

Introduction

Helicoverpa armigera and *H. punctigera* are major insect pests on summer crops in Australia. Although much research has been done on the ecology of these species (Zalucki et al. 1986), there is only limited knowledge of their genetics (Daly and Gregg 1985) and most of this concerns the genetics of insecticide resistance in *H. armigera* (e.g., Daly et al. 1988). Further genetic research is impeded by the lack of cytological studies and linkage maps. The latter would be useful for determining genetic linkage between genes conferring resistance to pesticides or biocontrol agents.

Until recently, *Helicoverpa* was a subgenus of *Heliothis* (Matthews 1987). Both genera are in the subfamily Heliothinae. Some cytological information is avilable for the North American pest *Heliothis virescens*, which has 31 pairs of chromosomes (Chen and Graves 1970). Linkage maps are currently being constructed for *Heliothis virescens* by using electrophoretic markers (D. Heckel, personal communication). The chromosomes of Lepidoptera are generally thought to be holocentric (White 1973).

The mapping of chromosome markers is greatly simplified if there is no crossing-over of genetic material between homologous chromosomes in one sex during meiosis because only one marker is needed to define each chromosome as a linkage group. If two loci are tested by crossing double heterozygous female with a double homozygous male, the two loci will show complete linkage if they are on the same chromosome, or complete independence if on different chromosomes.

It is generally presumed that the absence of genetic crossing-over, inferred from genetics studies, is associated with the absence of chiasmata: the absence of physical crossing of chromatids from different homologues in a bivalent, observed by cytological examination. This has been well documented for the achiasmatic meiosis of male *Drosophila* species (White 1973). In Lepidoptera and *Drosophila* species it is the heterogametic sex that is achiasmatic.

Achiasmatic female meiosis has been observed, by cytological studies, in 11 families of Lepidoptera and in the closely related order Trichoptera (Table 1). Additionally, limited cytological information suggests that female meiosis is probably achiasmatic in several species of Pyralidae and Tortricidae (Suomalainen 1969*a*, 1971) and in *Erebia medusa* (Nymphalidae) (Federley 1938). Although primitive and advanced families are represented, this is only a small sample of the Lepidoptera, currently classified into 127 families (E. Nielsen and I. Common, personal communication). The only report of achiasmatic female meiosis from the large family Noctuidae is for species of *Euxoa* in the subfamily Noctuinae (Fontana 1976). There are no reports from the subfamily Heliothinae. Cytological and genetic studies have confirmed that the absence of chiasmata is accompanied by the absence of crossing-over in four Lepidopteran species: *Ephestia kuehniella* (Pyralidae) (Traut 1977), two species of *Heliconius* (Nymphalidae) (Turner and Sheppard 1975), and *Bombyx mori* (Bombycidae) (Sturtevant 1915; Turner 1979).

In addition, there have been no substantiated reports of chiasmatic female meiosis in the Lepidoptera. Suomalainen (1953) claimed that females of the genus *Cidaria* (Geometridae) had bivalents with single terminal chiasmata at metaphase I, but with improved techniques (Suomalainen 1965) he subsequently discounted this and showed that female meiosis was achiasmatic.

Since achiasmatic female meiosis has been confirmed in only a small proportion of Lepidoptera, it is important to

TABLE 1. Reports of achiasmatic female meiosis from cytological studies in Lepidoptera and Trichoptera

Family	Species	Reference
Lepidoptera		
Micropterigidae	*Micropteryx aureatella*	Suomalainen 1969*b*
Eriocraniidae	*Eriocrania semipurpurella*	Suomalainen 1969*b*
Hepialidae	*Hepialus hecta*	Suomalainen 1969*b*
Incurvariidae	*Incurvaria pectinea*	Suomalainen 1969*b*
Pyralidae	*Ectomylois ceratoniae*	Morag et al. 1983
	Ephestia kuehniella	Traut 1977
Geometridae	*Cideria* species	Suomalainen 1965
Nymphalidae	*Charex jasius*	Trentini and Marini 1986
	Agraulis vanillae	Suomalainen et al. 1973
	Dryadula phaetusa	Suomalainen et al. 1973
	Heliconius (7 species)	Suomalainen et al. 1973
Bombycidae	*Bombyx mori*	Maeda 1939
	Bombyx mori and *B. mandarina*	Murakami and Imai 1974
Saturniidae	*Philosamia ricini*	Narang and Gupta 1979
	Antheraea compta and *A. assamensis*	Gupta and Narang 1981
Sphingidae	*Sphinx ligustri*	Nokkala 1987
Noctuidae	*Euxoa* (6 species)	Fontana 1976
Trichoptera	*Limnophilus decipiens* and *L. borealis*	Suomalainen 1966

investigate this aspect for other species that are to be used for linkage studies. For example, in *Drosophila* one species, *D. ananassae*, has been shown to have recombination in males (Hinton 1970) and is the only exception that has been found in this genus.

This paper reports on the karyotype of *H. armigera* and *H. punctigera* and the absence of chiasmata in female meiosis of both species.

Materials and methods

One *H. punctigera* strain and several *H. armigera* strains were examined. The *H. punctigera* strain and one *H. armigera* strain were derived from composite field collections in Queensland, Australia, and maintained in laboratory culture for 15 and 9 years, respectively, by R. E. Teakle (personal communication). A variety of *H. armigera* strains were derived from composite field collections from the Emerald Irrigation Area, Queensland, and the Namoi Valley, New South Wales, Australia, during 1985 and 1986. The insects were reared at 25°C by the procedure of Teakle and Jensen (1985). Under these conditions, eggs hatch after 3 days, there are five or six larval instars, and pupation occurs 15–17 days after egg hatch.

Mitosis

Eggs 1 to 1.5 days old are most suitable for mitotic metaphase preparations. This stage of development can be identified by the pale ring of yellow pigment that develops on the egg membrane and that darkens to brown at 2 days after laying. Live embryos were dissected from eggs and incubated in 0.5% colchicine in insect saline (0.7% NaCl and 0.02% $CaCl_2$ in water) for 1 h at room temperature. This solution was replaced by two changes of fixative (ethanol – acetic acid (3:1)) and left for 1–2 h.

After fixation, embryos were transferred to a microscope slide in a drop of 60% acetic acid and mashed with a flat-ended rod, and the drop was moved around the slide until all the liquid had evaporated. Thoroughly air-dried slides were stained with 10% Giemsa in Sørensen buffer (0.1% KH_2PO_4 and 0.082% Na_2HPO_4 in water, pH 6.8) for 20 min, rinsed with buffer, followed by water, and then dried. Slides were mounted with DePex. Nuclei were photographed at ×2000.

Male meiosis

Spermatocytes at metaphase I can be found in the testes of final instar larval males from about 13 days old, but are most abundant in 14- or 15-day-old larvae. Testes were dissected from live larvae and fixed for at least 1 h or stored in fixative at 4°C for up to 3 weeks. Air-dried slides were prepared and stained using the method described above.

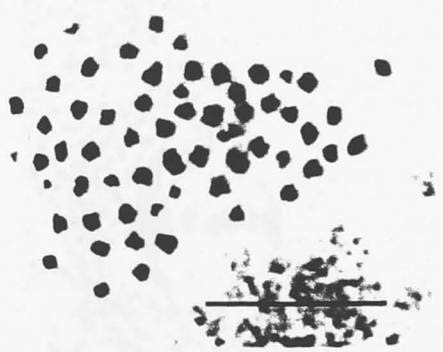


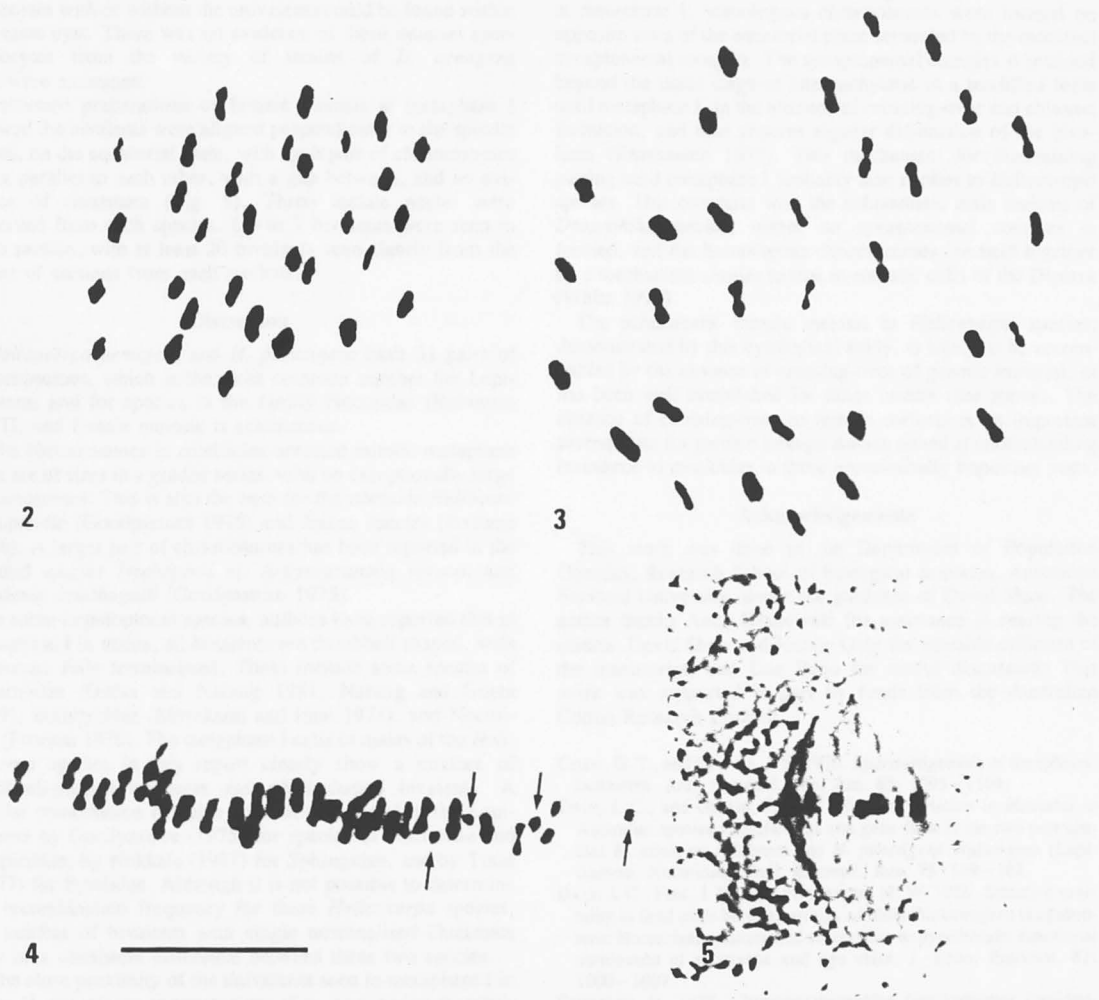
FIG. 1. Mitotic metaphase in *H. armigera* with 62 chromosomes. Bar represents 10 μm.

Female meiosis

As a result of the large amount of yolk in an egg, it was not possible to observe female meiosis by the air-dried method used for male meiosis, so observations were made using sectioned material. During oogenesis, the chromosomes are suspended at metaphase I in the mature ovules until fertilisation. Whole mature ovaries were dissected under insect saline from adult females 2 days after emergence and fixed for at least 1 h. The ovaries were then dehydrated in ethanol and embedded in LR-White resin (London Resin Co.). Sections 1.5 μm thick were cut and transferred onto precleaned slides in a drop of water and dried on a warm hot plate for at least 1 day. Slides were stained with 20% Giemsa for 15 min, dried, and mounted in DePex. Sections were also prepared from fixed testes for a direct comparison of male and female meiosis.

Results

At least 20 clearly resolved mitotic cells at metaphase were



FIGS. 2–5. Meiotic metaphase I in *Helicoverpa* spp. Fig. 2. *H. armigera* spermatocyte with 31 bivalents. Fig. 3. *H. punctigera* spermatocyte with 31 bivalents. Fig. 4. *H. punctigera* spermatocyte with 29 bivalents and 4 univalents (arrowed). Lateral view of metaphase plate. Fig. 5. *H. punctigera* oocyte. Lateral view of metaphase plate. Figs. 2–4. Air-dried preparation. Fig. 5. Sectioned preparation. Bar represents 10 μm for all figures.

examined from at least 10 embryos for both species. The karyotypes of *H. armigera* and *H. punctigera* were found to be similar. At mitotic metaphase there were 62 chromosomes in a graded series of sizes, between 0.5 and 1 μm long, but individual pairs could not be differentiated (Fig. 1). Since the sex ratio in *Helicoverpa* species larvae is approximately 1:1, it is likely that mitosis was observed from both male and female embryos, but no dimorphism was observed. The haploid number of 31 was confirmed by counts from 60 spermatocytes at meiotic metaphase I from 16 male *H. armigera* (Fig. 2) and 110 spermatocytes from 9 male *H. punctigera* (Fig. 3).

At metaphase I in the male, both air-dried slides and sections showed the bivalents were oriented parallel to the spindle fibres (Fig. 4). The bivalents were clearly chiasmate, showing a mixture of dumbbell and cross shapes. Although the shape of the bivalents was sometimes difficult to determine, it was estimated from air-dried preparations that between 15 and 21 bivalents per cell from *H. punctigera* ($\bar{x} = 18.3$, $SD = 1.1$, $n = 20$) were dumbbell shaped, but only 8–12 per cell from *H. armigera* ($\bar{x} = 10.3$, $SD = 1.8$, $n = 8$). The dumbbell shapes represent single terminal chiasmata. Whether the cross-shaped bivalents represent a single nonterminal chiasma or chiasmata terminalised at both ends could not be determined because earlier stages of meiosis were not observed.

Some unusual spermatocytes were observed from *H. punctigera*. Eighteen percent of spermatocytes at meiotic metaphase I contained 30 bivalents and 2 univalents. The two univalents were always close to each other on the metaphase plate. In one spermatocyte, 4 univalents and 29 bivalents were observed

(Fig. 4). The remaining 82% of nuclei had 31 bivalents. Spermatocytes with or without the univalents could be found within the same cyst. There was no evidence of these unusual spermatocytes from the variety of strains of *H. armigera* that were examined.

Sectioned preparations of female meiosis at metaphase I showed the bivalents were aligned perpendicular to the spindle fibres, on the equatorial plate, with each pair of chromosomes lying parallel to each other, with a gap between, and no evidence of chiasmata (Fig. 5). Three female nuclei were observed from each species. Up to 7 bivalents were seen in each section, with at least 20 bivalents seen clearly from the series of sections from each nucleus.

Discussion

Helicoverpa armigera and *H. punctigera* have 31 pairs of chromosomes, which is the most common number for Lepidoptera, and for species in the family Noctuidae (Robinson 1971), and female meiosis is achiasmatic.

The chromosomes in colchicine-arrested mitotic metaphase cells are of sizes in a graded series, with no exceptionally large chromosomes. This is also the case for the noctuids *Heliocontia apicella* (Goodpasture 1975) and *Euxoa* species (Fontana 1976). A larger pair of chromosomes has been reported in the noctuid species *Trichoplusi ni*, *Argyrogramma veruca*, and *Prodenia ornithogalli* (Goodpasture 1975).

In some Lepidopteran species, authors have reported that at metaphase I in males, all bivalents are dumbbell shaped, with chiasmata fully terminalised. These include some species of Saturniidae (Gupta and Narang 1981; Narang and Gupta 1979), Bombycidae (Murakami and Imai 1974), and Noctuidae (Fontana 1976). The metaphase I cells of males of the *Helicoverpa* species in this report clearly show a mixture of dumbbell-shaped bivalents and cross-shaped bivalents. A similar combination has also been demonstrated in the Lepidoptera by Goodpasture (1975) for species of Noctuidae and Hespiriidae, by Nokkala (1987) for Sphingidae, and by Traut (1977) for Pyralidae. Although it is not possible to determine the recombination frequency for these *Helicoverpa* species, the number of bivalents with single terminalised chiasmata may be a consistent difference between these two species.

The close proximity of the univalents seen in metaphase I in some *H. punctigera* spermatocytes (Fig. 4) suggests that they had recently been joined as bivalents and are the result of precocious separation. Precocious separation of bivalents has been reported from some field-caught specimens of *H. armigera* from Emerald, Queensland, in 1984 (C. Goodpasture, personal communication). Reports of this feature from other Lepidoptera are from *Hoplotarche lunana* (Noctuidae) and *Catopsilia phyranthe* (Pieridae) (Gupta 1964), and *Philosamia ricini* (Saturniidae) (Srivastava and Gupta 1962), where the presence of the univalents was attributed to either a lack of chiasma formation or a precocious separation of the homologues of the bivalents. Nuclei at the second meiotic division were not observed in *H. punctigera*. However, counts from metaphase II plates from *Hoplotarche lunana*, *C. phyranthe*, and *P. ricini* were always of the normal haploid number, indicating that meiosis proceeded normally (Gupta 1964; Srivastava and Gupta 1962).

Electron micrographs of thin sections from oocytes of the silkmoth *Bombyx mori* (Rasmussen 1977) illustrate that the achiasmatic female meiosis in *B. mori* is similar to that observed in *H. armigera* and *H. punctigera* (Fig. 5). In *B. mori* at metaphase I, homologous chromosomes were located on opposite sides of the equatorial plane separated by the modified synaptonemal complex. The synaptonemal complex is retained beyond the usual stage of late pachytene in a modified form until metaphase I, in the absence of crossing-over and chiasma formation, and thus ensures regular disjunction of the bivalents (Rasmussen 1977). This mechanism for maintaining pairing until metaphase I probably also applies to *Helicoverpa* species. This contrasts with the achiasmatic male meiosis of *Drosophila* species, where no synaptonemal complex is formed, and the homologous chromosomes are held together by a mechanism similar to that in somatic cells of the Diptera (White 1973).

The achiasmatic female meiosis in *Helicoverpa* species, demonstrated by this cytological study, is likely to be accompanied by the absence of crossing-over of genetic material, as has been well established for other insects (see above). The absence of crossing-over in female meiosis is an important prerequisite for genetic linkage studies aimed at understanding resistance to pesticides in these economically important pests.

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