Morphological and molecular studies of tortricid moths of economic importance to the South African fruit industry

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Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: ………………………..
Date: …………………………….

Abstract

Six tortricid species are of major economic importance to the South African fruit industry. They are the codling moth *Cydia pomonella*, the oriental fruit moth *Grapholita molesta*, the false codling moth *Thaumatotibia leucotreta*, the macadamia nut borer *T. batrachopa*, the litchi moth *Cryptophlebia peltastica* and the carnation worm *Epichoristodes acerbella*. For phytosanitary purposes and to aid the management of population levels of the aforementioned species, their identities at species and population level were investigated using morphological and molecular genetic techniques. Morphological characteristics were used to distinguish and differentiate between the final instar larvae and pupae of the six species. For this purpose the morphology of the final instar larvae and pupae of the Afrotropical species *T. leucotreta*, *T. batrachopa*, *Cr. peltastica* and *E. acerbella* was described and illustrated using line drawings and scanning electron micrographs. Taxonomic characters found to be useful for the identification of the larvae were the presence and structure of the anal comb and the number and arrangement of crochets on the prolegs. The pupae could be distinguished based on the presence or absence of a distinct cremaster, the shape of the spiracle, the position of the setae on the anal rise, the structure of the mouthparts and the length of the procoxa in relation to that of the protarsus. These characters were used to develop keys to distinguish between the tortricid species occurring on tropical and subtropical fruit (*T. leucotreta*, *T. batrachopa* and *Cr. peltastica*) and deciduous fruit (*E. acerbella*, *C. pomonella*, *G. molesta* and *T. leucotreta*). At population level, molecular techniques were employed to compare geographic populations of each of the six species. Amplified fragment length polymorphism (AFLP) analysis with five selective primer pairs was used to investigate genetic diversity. In addition, host populations of species were compared where relevant. No evidence was found to suggest that populations from different hosts were genetically differentiated. However, geographic populations were found to be genetically distinct in each of the six species, with extensive genetic divergence apparent over local geographic scales and significantly high estimates of population differentiation ranging between $G_{st} = 0.2625$ and 0.3778. Factors influencing the genetic population structure of the six species were investigated by comparing the amount and distribution of genetic variation between oligophagous and polyphagous species as well as introduced and native species. Results indicated that host range and population history did not have a major effect on population genetic structure. It was therefore suggested that other factors such as limited dispersal were responsible for the extensive genetic divergence observed between geographic populations of each of the six tortricid species. These results should be incorporated into existing pest management programs and taken into consideration when designing new control strategies. This is the first report of its kind to identify, with a high level of accuracy, the aforementioned tortricids and the first to determine the population genetic structure of these species.

Opsomming

Ses tortrisiede spesies is van ekonomiese belang vir die Suid-Afrikaanse vrugtebedryf. Hulle is die kodlingmot *Cydia pomonella*, die Oosterse vrugtemot *Grapholita molesta*, die valskodlingmot *Thaumatotibia leucotreta*, die makadamianeutboorder *T. batrachopa*, die lietsjiemot *Cryptophlebia peltastica* en die peerbladroller *Epichoristodes acerbella.* Vir fitosanitêre doeleindes en die beheer van hierdie ses spesies is hulle identiteit op spesies- en populasievlak ondersoek deur gebruik te maak van morfologiese en molekulêre genetiese tegnieke. Morfologiese kenmerke is gebruik om te onderskei tussen die finale instar larwes en papies van die ses spesies. Vir hierdie doel is die morfologie van die finale instar larwes en papies van die Afrotropiese species *T. leucotreta*, *T. batrachopa*, *Cr. peltastica* en *E. acerbella* beskryf deur gebruik te maak van lyntekeninge en skandeer-elektronmikrograwe. Die teenwoordigheid en struktuur van die anale kam en die hoeveelheid en rangskikking van die buikpoothakies was goeie kenmerke vir die uitkenning van die larwes. Die papies kon onderskei word deur gebruik te maak van die aanwesigheid van ’n duidelike kremaster, die vorm van die spirakula, die posisie van die setae op die anale bult, die struktuur van die monddele en die lengte van die procoxa teenoor die van die protarsus. Hierdie kenmerke is gebruik om sleutels te ontwikkel om te onderskei tussen die tortrisiede spesies wat op tropiese en subtropiese gewasse (*T. leucotreta*, *T. batrachopa* and *Cr. peltastica*) en bladwisselende gewasse (*E. acerbella*, *C. pomonella*, *G. molesta* and *T. leucotreta*) voorkom. Op populasievlak is molekulêre tegnieke ingespan om geografiese populasies van elkeen van die ses spesies te vergelyk. Geamplifiseerde fragment-lengte polimorfisme (AFLP) ontledings met vyf selektiewe inleier pare is gebruik om genetiese diversiteit te ondersoek. Waar toepaslik, is gasheerpopulasies van spesies vergelyk, maar geen verskil is tussen sulke populasies gevind nie. In teenstelling hiermee is geografiese populasies van elkeen van die ses spesies geneties onderskeibaar met uitgebreide genetiese verskille merkbaar oor klein geografiese afstande en hoë waardes van populasie differensiasie is waargeneem (G_{st} = 0.2625 tot 0.3778). Faktore wat die genetiese populasiestruktuur van die ses spesies beïnvloed, is ondersoek deur die hoeveelheid en verspreiding van genetiese variasie tussen oligo- en polifage spesies sowel as indringer en inheemse spesies te vergelyk. Resultate het getoon dat gasheerreeks en populasiegeskiedenis nie ’n groot impak op populasiestruktuur gehad het nie. Daar is dus voorgestel dat faktore soos swak verspreidingsvermoë verantwoordelik is vir die uitgebreide genetiese divergensie wat tussen geografiese populasies gevind is. Hierdie resultate behoort in ag geneem te word by bestaande plaagbestuurprogramme en die ontwerp van nuwe beheerstrategieë. Hierdie is die eerste verslag van sy soort wat, met ’n hoë vlak van akkuraatheid, die genoemde ses tortrisied spesies identifiseer en die eerste wat die genetiese populasiestruktuur van hierdie spesies bepaal het.

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List of abbreviations and symbols

γ	Gamma
°C	Degrees centigrade
μCi	Microcurie
μl	Microlitre
μM	Micromolar
A	Adenosine
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
C	Cytosine
CTAB	N-acetyl-N,N,N-trimethyl-ammonium bromide
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
G	Guanine
HCl	Hydrochloric acid
KOH	Potassium hydroxide
kV	Kilovolts
M	Molar
$MgCl_2$	Magnesium chloride
Mm	Millimetre
mM	Millimolar
nA	Nanoamperes
ng	Nanogram
Pa	Pascals
PCOA	Principal co-ordinate analysis
Pmol (es)	Picomole (s)
s	Plural form of setae e.g. D1s
T	Thymine
TE buffer	Tris EDTA buffer
TRIS	2-amino-2(hydroxymethyl)-1,3-propandiol

UPGMA	Unweighted pair group means arithmetic
V	Volts
W	Watts

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CHAPTER 1

Introduction

The Tortricoidea comprises a single family, the Tortricidae (Horak & Brown 1991), and contains some of the most important agricultural pest species in the world. Tortricid larvae are typically concealed feeders, often boring into fruit and stems and spinning and rolling leaves in which they develop. In South Africa six tortricid species are of major economic importance to the fruit industry, namely. the Afrotropical species false codling moth *Thaumatotibia leucotreta* (Meyrick), macadamia nut borer *T. batrachopa* (Meyrick), litchi moth *Cryptophlebia peltastica* (Meyrick) and carnation worm *Epichoristodes acerbella* (Walker) and the alien invasive oriental fruit moth *Grapholita molesta* (Busck) and codling moth *Cydia pomonella* (L.) (Plate 1.1) (Deciduous Fruit Grower 1997, Giliomee & Riedl 1998, Newton 1998, Blomefield & Barnes 2000, de Villiers 2001a,b,c). The larvae of these species cause extensive damage to a range of cultivated crops and may also feed on wild host species. Management of their population levels is often erratic or ineffective, which may partly be due to the fact that they often cannot be identified accurately and that aspects of their ecology are poorly understood. Therefore, the current study aimed to address these shortcomings by focussing on (1) the identity of the immature stages (fully grown larvae and pupae) and (2) the population genetic structure of each of the six species.

1. Identification of immature stages

The correct identity of an insect pest is of primary importance both for phytosanitary purposes and the management of its field population levels. The adults of the six economically important tortricid species can readily be identified using morphological and taxonomic characters. However, the identification of their immature stages is often problematic since many of the larvae and pupae are morphologically rather similar and often misidentified. Thus, methods for distinguishing and differentiating between the final instar larvae (the instar most frequently intercepted by quarantine stations) and pupae of the six species are necessary. Preferably, these methods should be accurate, rapid and economical. A morphological system of identification fits these criteria, requiring only descriptions of the species to be identified. However, accurate descriptions of the immature stages, particularly for the Afrotropical species, are lacking. Therefore, one of the aims of this study was to (i) provide a system of identification for use by quarantine and pest control officers by describing and illustrating the morphology of the final instar larvae and pupae of the economically important tortricid species in South Africa and (ii) develop appropriate keys to distinguish and differentiate between the different species.

2. Analysis of population genetic structure

Analysis of the population genetic structure of an organism may provide valuable insight into ecological aspects such as gene flow between populations and dispersal dynamics (Bohonak 1999). Since many practices used to control phytophagous insect populations are affected by these factors, analysis of population genetic structure may be of particular value for insect agricultural pests. Insight gained from such studies may be useful for the design of new control strategies and more effective use of existing practices. Analysis of population genetic structure may thus ultimately aid the management of population levels of the economically important tortricids in South Africa and was therefore considered to be another important aim of this study. Amplified fragment length polymorphism (AFLP) analysis (Vos *et al*. 1995) was chosen for examination of tortricid populations since this marker permits co-amplification of high numbers of restriction fragments, which allows for greater resolution of the determination of genetic variation (Mendelson & Shaw 2005). Of the six tortricid species considered, only *C. pomonella* has been analyzed previously at population level using molecular markers (Pashley & Bush 1979, Bués *&* Toubon 1992). Therefore, the fact that AFLP analysis can be conducted without prior knowledge of the nucleotide sequence of the genome being studied was a further advantage. For this reason AFLP analysis was used to determine genetic diversity (the amount of genetic variation) and genetic differentiation (the distribution of genetic variation among populations and the amount of genetic variation between pairs of populations) of populations of the tortricids of major economic importance in South Africa to gain further insight into their ecology and eventual control.

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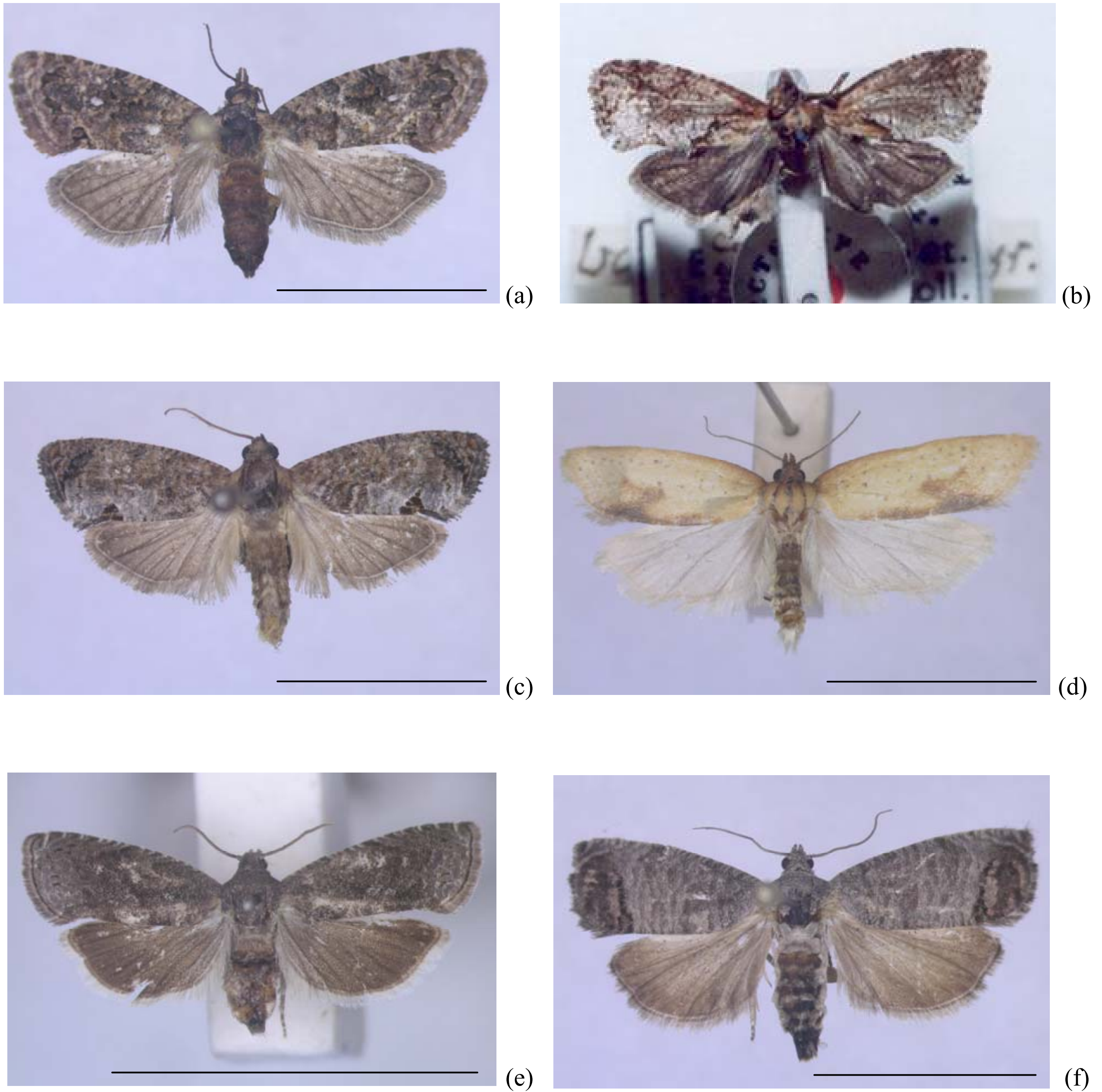

Plate 1.1. Tortricid moths of major economic importance to the South African fruit industry. **a**, *Thaumatotibia leucotreta* (Meyrick); **b**, *T. batrachopa* (Meyrick) (lectotype)*; **c**, *Cryptophlebia peltastica* (Meyrick); **d**, *Epichoristodes acerbella* (Walker); **e**, *Grapholita molesta* (Busck); **f**, *Cydia pomonella* (L.). Scale bar = 1cm. *Photograph H. Geertsema, courtesy of the Natural History Museum (British Museum), London.

CHAPTER 2

Comparative morphological analysis of the final instar larvae of Tortricidae (Lepidoptera) of economic importance on tropical and subtropical fruit tree crops in South Africa

2.1 Abstract

The final instar larvae of the false codling moth *Thaumatotibia leucotreta*, the macadamia nut borer *T. batrachopa* and the litchi moth *Cryptophlebia peltastica* are described and illustrated. Diagnostic characters are given for each of the species and a key included to facilitate identification.

2.2 Introduction

Members of the family Tortricidae form an important component of the insect complex attacking tropical and subtropical fruit worldwide (Jones 1995). In South Africa three tortricid species are of economic importance on tropical and subtropical fruit, namely the false codling moth *Thaumatotibia leucotreta* (Meyrick), the macadamia nut borer *T. batrachopa* (Meyrick) and the litchi moth *Cryptophlebia peltastica* (Meyrick). The host ranges of the three species overlap on crops such as macadamias and litchis, which are the only cultivated crops on which *T. batrachopa* and *C. peltastica* occur (de Villiers 2001a,b,c). However, *T. leucotreta* also occurs as a pest of a wide variety of cultivated crops such as avocados, guavas, pecans, persimmons, apples, pears and peaches (Blomefield 1989, Newton 1998). Therefore, accurate descriptions of their morphology are essential for phytosanitary purposes as well as effective control. However, only the taxonomic descriptions of the adult stages of all three species have been provided (Meyrick 1908, Meyrick 1921, Bradley 1953, Swart 1966, Krüger 1998), restricting identification to the adult level. Despite the economic importance of the damage caused by the larvae, detailed descriptions of the immature stages are lacking for *T. batrachopa* and *C. peltastica* although the latter was considered by McGeogh & Krüger (1994). Stofberg (1948) described the morphology of the final instar larvae of *T. leucotreta* but advances in technology have enabled the study of various morphological structures in more detail with a greater degree of accuracy. The aims of this study were therefore to (1) describe the morphology of the final instar larvae of *T. leucotreta*, *T. batrachopa* and *C. peltastica*, and (2) develop keys to distinguish between the three species to permit unambiguous identification.

2.3 Material and methods

2.3.1 Insect material

Specimens of *T. leucotreta* were obtained by donation from an established laboratory colony (Ceder Biocontrol, Citrusdal) whereas *T. batrachopa* specimens were obtained by field collection (Institute for Tropical & Subtropical Crops, Nelspruit, 2003). Specimens of *C. peltastica* were obtained by the establishment of a laboratory colony in the Department of Entomology at Stellenbosch University from material collected at the Institute for Tropical & Subtropical Crops, Nelspruit during 2003. Voucher specimens were deposited in the museum of the Department of Entomology, University of Stellenbosch.

2.3.2 Light microscopy

Fully hydrated larval material was used for examination. Transparent preparations of larval material were also made by conventional treatment using 10% KOH to remove the gut contents, neutralizing material in 10% HCl and staining using acid fuchsin. Material was dried progressively using an alcohol dehydration series and mounted permanently on microscope slides. Measurements were taken using an ocular micrometer and digital photomicrographs generated using a Wild M8 stereo microscope with a 5 mega pixel Nikon Coolpix camera to facilitate the production of line drawings.

2.3.3 Scanning electron microscopy

Larval specimens were examined uncoated and fully hydrated using a Leo® 1430VP SEM (LEO Electron Microscopy, Thornwood, New York) with variable pressure mode. In this mode, back-scattered electrons were used to create the image. The beam conditions were an accelerating voltage of 25 kV and a spotsize of 350, corresponding to approximately 1.5 nA. Vacuum pressure in the chamber was set to a minimum of 50 Pa.

2.3.4 Nomenclature

Heinrich's (1916) system of nomenclature was used for the head setae. The setae of the thorax and abdomen were named according to Hinton (1946) and those of the anal shield according to MacKay (1959).

2.4 Results

2.4.1 *Thaumatotibia leucotreta* (Fig. 2.1, Plate 2.1)

2.4.1.1 General

Larva slender, elongate, cream to light red. Integument rugose. Setal pinacula easily observed, darker than body colour; spinulation of integument easily apparent, setae moderately long. Head yellowish brown with dark pigmentation at ocellar area and postgenal juncture. Prothoracic and anal shields distinct, medium brown with darker patches due to medium and extensive sclerotization.

2.4.1.2 Head (Fig. 2.1 a-e; Plate 2.1 a,c)

Hypognathous, dorsal surface flattish and broad. Average width prior to pupation, based on 29 specimens, 1.31 mm. Vertical angle acute. Adfrontals tapering anteriorly and extending to vertical angle. P^1 closer to Adf^2 than F^1. Ocellar areas rounded. Stemmata approximately equal in size, irregularly rounded. Stemma II closer to stemma I than III. Stemma III and IV situated close together. Stemma V closer to stemma IV than VI. Stemma V separated from VI by distance nearly equal to half diameter of stemma V. O^1 equidistant from stemma II and III. A^2 closer to A^1 than A^3. A^3 closer to L^1 than A^2. Line joining O^1 and A^1 closer to stemma III than stemma II. Line joining O^1 and L^1 through median of stemma I. Mandible with five teeth, outer three large, usually acuminate although second or third often flattened, fourth smaller and flattened and fifth straight-edged. Antenna elongate, shorter, less robust than labial palps, with long terminal seta. Distal end of spinneret rounded, about eight times longer than wide.

2.4.1.3 Thorax (Fig. 2.1 f,j; Plate 2.1 b,d)

Prothoracic shield with anterior lateral margin obtuse, slightly concave, curved at about one-third of its length and curved convexly towards the mid-line, lateral margin fairly straight and posterior margin evenly rounded towards mid-line. On prothorax, spiracle circular; L1 equidistant from L2 and L3, L1 more or less in a straight line with L2 and L3, SD1 equidistant from XD2 and SD2. On meso- and metathorax D1 dorsal to D2. Thoracic claws small, curved.

2.4.1.4 Abdomen (Fig 2.1 g-j)

Spiracles oval, small, seldom larger than setal bases. SD1 separated from spiracle by approximately 1.5 times diameter of spiracle. On segment 8 (A8) spiracle slightly anterior to midvertical line through segment; SD1 anteroventral to spiracle at about three times spiracle diameter. SV group on A1, 2, 7, 8 and 9 usually 3:3:2:2:1. On A9 D1 and SD1 on same pinaculum; L1 equidistant to L2 and L3.

Anal fork. Well developed, darkly pigmented, with 2-10 bluntly dentate prongs. Basal part of each prong strongly tapered dorsally, with width of the base nearly one-quarter length of tooth, upper levels of larger prongs medially at about same level.

Anal shield. Tapering posteriorly, evenly rounded along posterior margin, lateral margin acute, angled anteriorly, anterior margin broadly curved. L1s further apart than D1s. D2s half as long as L1s. D1s slightly closer to SD1s than to each other, D1s slightly shorter than SD1s.

Prolegs. Crochets of abdominal prolegs irregularly triordinal, 36-42 and 24-32 on anal prolegs. Anal prolegs with crochets absent in medial half.

2.4.2 *Thaumatotibia batrachopa* (Fig. 2.2, Plate 2.2)

2.4.2.1 General

Larva elongate, cream to grey green. Integument rugose. Setal pinacula moderately large, easily observed. Spinulation of integument conspicuous, spinules slender and darker than body colour. Head yellow brown with darker pigmentation at ocellar area. Prothoracic and anal shields yellow brown, lightly sclerotised with small patches of darker pigmentation. Thoracic legs medium brown.

2.4.2.2 Head (Fig. 2.2 a-e; Plate 2.2 a,c)

Hypognathous, dorsoventrally flattened. Vertical angle acute. Adfrontals extending to vertical angle, tapering anteriorly and posteriorly. P^1 closer to Adf^2 than F^1. Stemmata approximately equal in size, irregularly rounded. Stemma II equidistant from I and III. Stemma III and IV close together, IV closer to III than VI. Stemma V and VI separated by distance nearly equal to diameter of V. O^1 equidistant from II and III. A^2 closer to A^1 than A^3. A^3 closer to L1 than A^2. Line through O^1 and A^2 equidistant from stemma II and III. Lines joining O^1 and A^1 and O^1 and A^3 nearly right angled. A^1, A^2, A^3 and L^1 on regular rounded arc. Mandible with five teeth, outer three pointed, second largest, fourth smaller and fifth smallest and straight edged. Antenna elongate with long terminal seta, shorter, less robust than labial palpus.

2.4.2.3 Thorax (Fig. 2.2 f,j; Plate 2.2 b,d)

Prothoracic shield with anterior lateral margin obtuse, slightly concave curved about one-third its length, curved convexly towards mid-line, lateral margin fairly straight, posterior margin evenly rounded towards mid-line. On prothorax, spiracle circular, L1, L2 and L3 in straight line, SD1 equidistant from XD2 and SD2. On meso- and metathorax D1 dorsal to D2. Thoracic claws curved.

2.4.2.4 Abdomen (Fig 2.2 g-j)

Spiracles circular, small, seldom larger than setal bases, SD1 usually separated by distance 1.5 times of spiracle except on A8. On A8 spiracle on mid-vertical line through segment; SD1 situated anteroventrally of spiracle at about twice its diameter. L2 anterodorsal to L1. SV group on 1, 2, 7, 8, 9 usually 3:3:2:2:1. On A9 D1 and SD1 on same pinaculum, L1 equidistant from L2 and L3.

Anal shield. Posteriorly rounded. L1s approximately same distance apart from each other as D1s. D2s slightly more than half as long as L1s. D1s somewhat closer to corresponding SD1s than to each other.

Anal fork. Well developed, darkly pigmented, with 5-8 prongs. Basal part of each prong strongly tapered dorsally, width of the base nearly one-quarter length of tooth, prongs merging into distinct medial structure.

Prolegs. Prolegs with 34-44, anal prolegs with 26-32 unevenly arranged triordinal crochets. Crochets of anal prolegs absent in medial half.

2.4.3 *Cryptophlebia peltastica* (Fig. 2.3, Plate 2.3)

2.4.3.1 General

Larva elongate, light pink to light red. Setal pinacula easily observed, with brown pigmentation; spinules slender, darker than body colour. Integument rugose. Head brown with darker pigmentation at ocellar areas. Prothoracic shield dark brown. Anal shield yellow brown with some medium brown pigmentation. Thoracic legs medium brown.

2.4.3.2 Head (Fig. 2.3 a-e; Plate 2.3 a-b)

Head hypognathous, dorso-ventrally flattened. Maximum width, based on 7 specimens, 2.05 mm. Adfrontals extending to vertical angle, tapering anteriorly. Vertical angle acute. P^1 closer to Adf^2 than F^1. Stemma II – VI rounded, approximately equal in size. Stemma I elongate, length nearly 1.5 times diameter of stemma II – VI. Stemma II closer to stemma I than III. Stemma IV closer to III than VI. Stemma IV and VI separated by distance slightly less than diameter of stemma IV. Stemma V and VI separated by distance roughly 1.5 times diameter of stemma V. O^1 closer to stemma III than stemma II. Line through O^1 and A^1 closer to stemma III than II, through O^1 and A^2 closer to stemma II than III and through O^1 and L^1 through the median of stemma I. A^1, A^3 and L^1 on regular shaped arc with A^2 dorsal. Mandibles with five teeth, outer three large, acuminate, second or third sometimes flattened, fourth smaller and flattened and fifth straight-edged. Distal end of spinneret rounded, about seven times longer than wide. Antenna elongate with long terminal seta, shorter, less robust than labial palpus.

2.4.3.3 Thorax (Fig. 2.3 f,j; Plate 2.3 c-d)

Prothoracic shield with anterior lateral margin obtuse, lateral margin fairly straight and posterior margin evenly rounded towards mid-line. On prothorax, spiracle circular, L1 equidistant from and in straight line with L2 and L3, SD1 slightly closer to SD2 than XD2. On meso- and metathorax D1 dorsal to D2. Thoracic claws curved.

2.4.3.4 Abdomen (Fig. 2.3 g-j)

Spiracles small, circular, seldom larger than setal bases, SD1 approximately 2.5 times its diameter from spiracle except on A8. On A8 spiracle slightly posterior to mid-vertical line through segment; SD1 situated anteroventrally to spiracle and about 1.5 times of spiracle diameter. L1 and L2 ventral to spiracle

and on A8 L2 slightly anterodorsal to L2. SV group on A1, 2, 7, 8, 9 usually 3:3:3:2:2. On A9 D1 and SD1 on same pinaculum; L1 equidistant to L2 and L3.

Anal shield. Anal shield rounded posteriorly. L1s slightly further lateral than D1s. D1s closer to corresponding SD1s than to each other and almost in a straight line.

Anal fork. Absent

Prolegs. Prolegs with 50-58, anal prolegs with 46-54 unevenly biordinal crochets, in anal prolegs absent in medial half.

2.5 Discussion

From a study of the morphology of final instar larvae of *T. leucotreta*, *T. batrachopa* and *C. peltastica*, numerous similarities can be noted. However, final instar larvae of *T. leucotreta* and *T. batrachopa* appeared closer to each other than *C. peltastica*, reflecting and justifying the recent removal of *T. leucotreta* and *T. batrachopa* from the genus *Cryptophlebia* (Komai 1999, Brown 2005). Morphological differences between larvae of the two genera in the current study include the absence of an anal comb, increased head width, differing stemma sizes and an increased number of setae on the SV group of the abdominal segments in *C. peltastica* relative to *T. leucotreta* and *T. batrachopa*. Based on these differences, a key was developed to distinguish between the larvae of the three species.

2.5.1 Key to species

1. Anal comb present ………………………………………………………………….……..……..2

   Anal comb absent; prolegs with unevenly biordinal crochets….…………….………*C. peltastica*

2. Crochets on prolegs unevenly triordinal in lateral half of arrangement; anal comb with medial prongs of even length …………………….………………………………………….…...*T. leucotreta*

   Crochets on prolegs unevenly biordinal; anal comb with prongs merging into distinct medial structure ……………………………..…………………………………………….*T. batrachopa*

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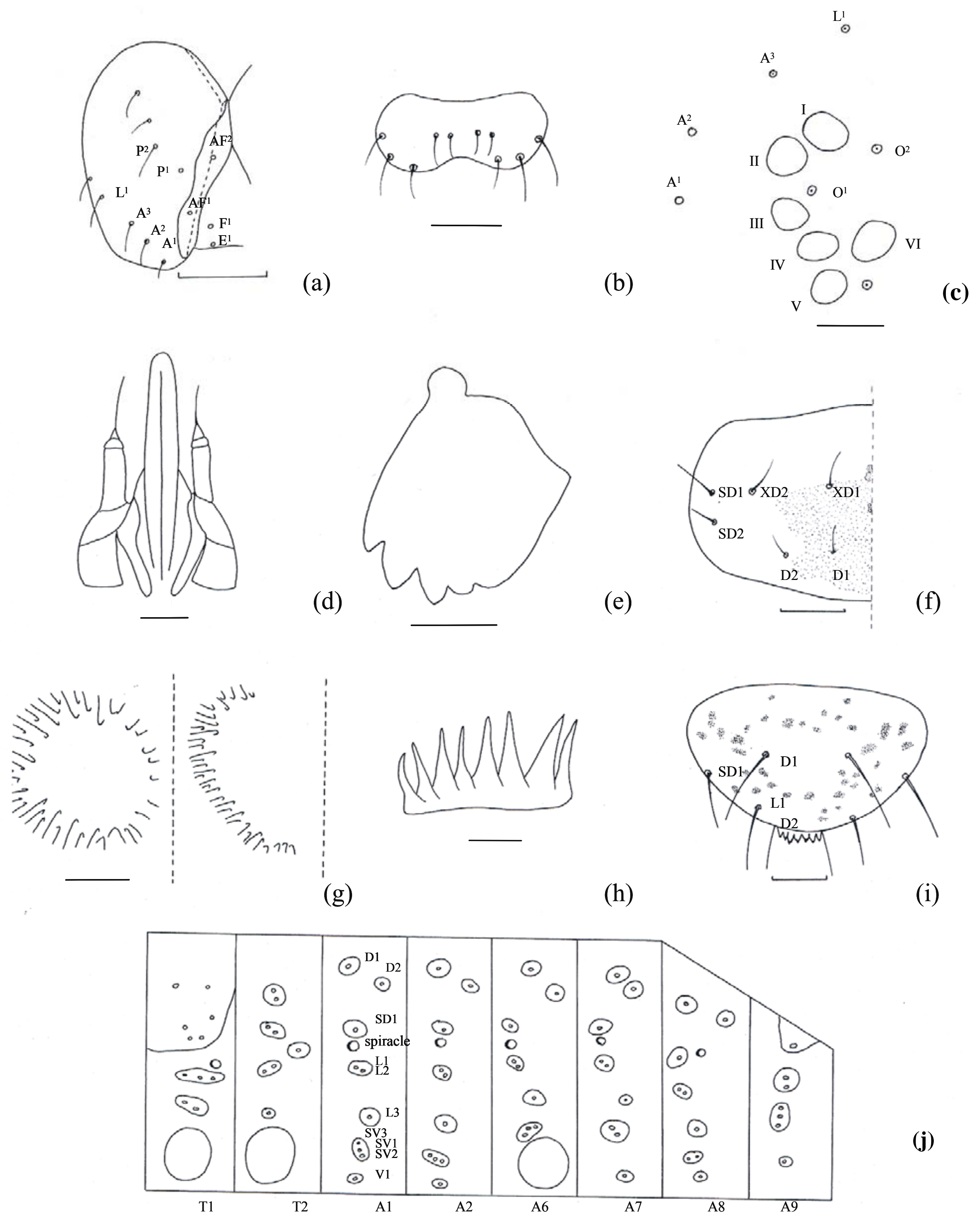


Fig. 2.1. *Thaumatotibia leucotreta* final instar larva. **a**, head (frontal aspect); **b**, labrum; **c**, stemmata; **d**, spinneret; **e**, mandible; **f**, prothoracic shield; **g**, crochets on ventral and anal prolegs; **h**, anal comb; **i**, anal shield; **j**, setal map. Scale bar: a, f, i = 1mm, b-e, g-h = 0.1mm.

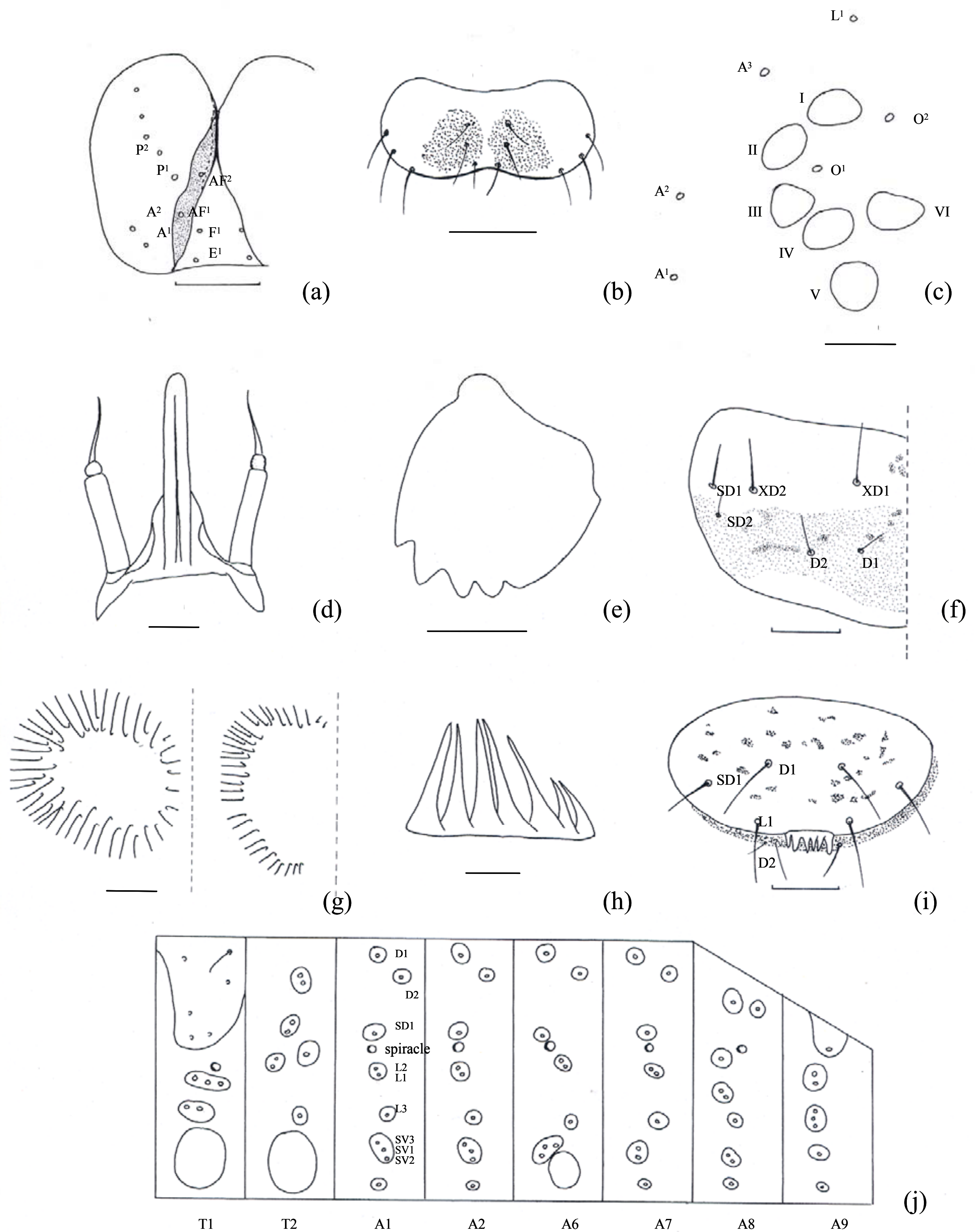


Fig. 2.2. *Thaumatotibia batrachopa* final instar larva. **a**, head (frontal aspect); **b**, labrum; **c**, stemmata; **d**, spinneret; **e**, mandible; **f**, prothoracic shield; **g**, crochets on ventral and anal prolegs; **h**, anal comb; **i**, anal shield; **j**, setal map. Scale bar: a, f, i = 1mm, b-e, g-h = 0.1mm.

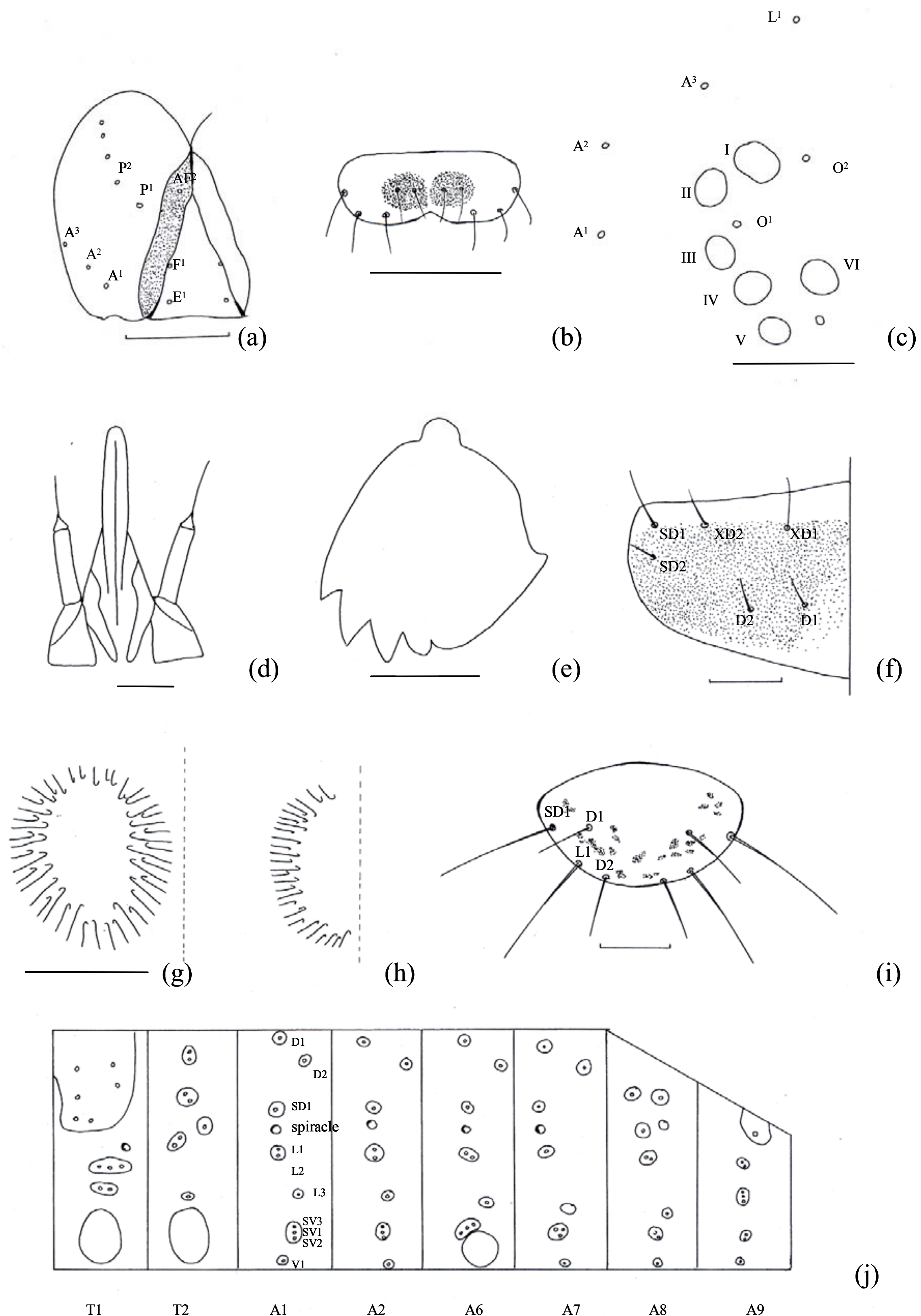
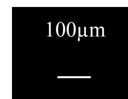
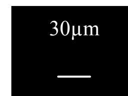


Fig. 2.3. *Cryptophlebia peltastica* final instar larva. **a**, head (frontal aspect); **b**, labrum; **c**, stemmata; **d**, spinneret; **e**, mandible; **f**, prothoracic shield; **g**, crochets on ventral prolegs; **h**, crochets on anal prolegs; **i**, anal shield; **j**, setal map. Scale bar: a, f, i = 1mm, b-e, g-h = 0.1mm.



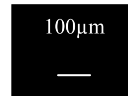
(a)



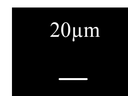
(b)

(c) (d)

Plate 2.1. *Thaumatotibia leucotreta* final instar larva. **a**, head (dorsal aspect); **b**, spiracle on prothorax; **c**, antenna; **d**, prothoracic leg.



(a)

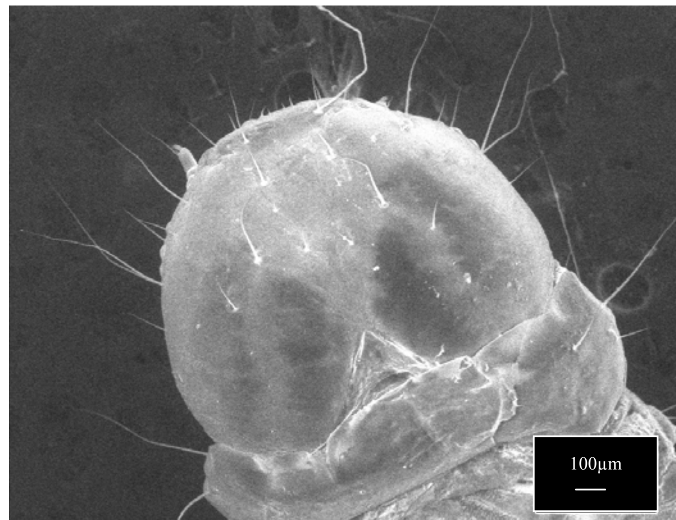


(b)

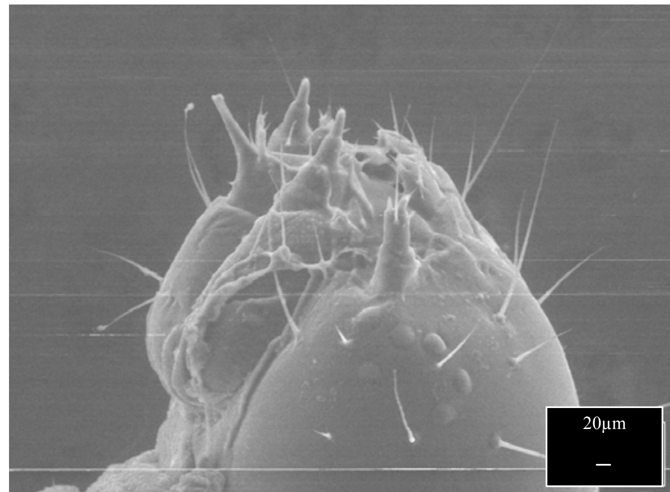
(c)

(d)

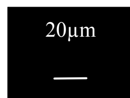
Plate 2.2. *Thaumatotibia batrachopa* final instar larva. **a**, head (dorsal aspect); **b**, spiracle on prothorax; **c**, antenna; **d**, prothoracic leg.



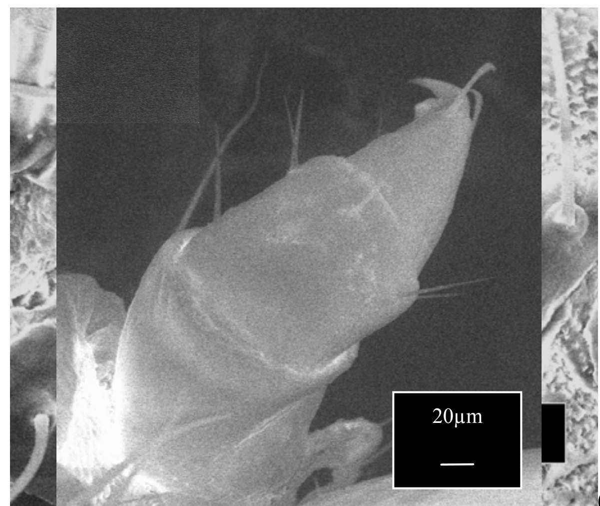
(a)



(b)



(c)



(d)

Plate 2.3. *Cryptophlebia peltastica* final instar larva. **a**, head (dorsal aspect); **b**, head (lateral aspect); **c**, spiracle on prothorax; **d**, prothoracic leg.

CHAPTER 3

Description of the final instar larva of *Epichoristodes acerbella* (Lepidoptera: Tortricidae) and comparison with other tortricid larvae of economic importance on deciduous fruit tree crops in South Africa

3.1 Abstract

The final instar larva of the carnation worm *Epichoristodes acerbella* is described and illustrated. The morphology of this species is compared to those of the three other tortricid species of major economic importance on deciduous fruit tree crops in South Africa, namely codling moth *Cydia pomonella*, oriental fruit moth *Grapholita molesta* and false codling moth *Thaumatotibia leucotreta*. A key is included to facilitate identification of the four species.

3.2 Introduction

The carnation worm *Epichoristodes acerbella* (Walker) (Lepidoptera: Tortricidae), which originates from South Africa, is one of the most damaging crop and flower pests of quarantine importance in this country and elsewhere. The larvae are polyphagous and able to feed on a variety of plant species, ranging from cultivated fruit trees such as pome fruit, stone fruit and grapes, ornamental plants such as *Dianthus* spp. and *Leucadendron* spp. and various weed species such as *Cryptostemma* spp., *Raphanus raphanistrum*, *Hypochoeris radicata* and *Rumex acetocella* (Basson 1960, Myburgh & Basson 1961, Annecke & Moran 1981, Wright 1995). *Epichoristodes acerbella* was accidentally introduced into various European countries, where it is known as a pest mainly of carnations (Zangheri & Cavalloro 1971, Nuzacci 1973, d'Aguilar & Deportes 1974, Sola 1974, Costa Seglar & Vives de Quadras 1976, Gabarra 1986). Despite the economic importance of the species, a detailed description of the morphology of the larva, the destructive stage, is lacking. In South African orchards of deciduous fruit tree crops, three morphologically similar larvae of tortricid species are found in addition to *E. acerbella*, namely the codling moth *Cydia pomonella* (L.), the oriental fruit moth *Grapholita molesta* (Busck), which both attack apples, pears and stone fruit, and the false codling moth *Thaumatotibia leucotreta* (Meyrick), which feeds on a wide variety of cultivated crops such as deciduous, subtropical and tropical fruit as well as citrus (Newton 1998). All four species may occur as internal feeders on fruit of deciduous trees and as such correct identification is essential both for effective control and phytosanitary purposes. The larvae of *C.*

pomonella and *G. molesta* have been described by MacKay (1959) and that of *T. leucotreta* by Stofberg (1948) and the current study (Chapter 2). The aims of this study were therefore to (1) describe the morphology of final instar larva of *E. acerbella* and (2) develop keys for distinguishing between *E. acerbella*, *C. pomonella*, *G. molesta* and *T. leucotreta* larvae.

3.3 Material and Methods

Specimens of all four species were obtained by donation from established laboratory colonies maintained at the Department of Entomology, University of Stellenbosch (*E. acerbella*), Hortec, Stellenbosch (*C. pomonella*), Agriculture and Agri-Food, Canada (*G. molesta*) and Ceder Biocontrol, Citrusdal (*T. leucotreta*). Light and scanning electron microscopy techniques used were as previously described (Chapter 2). The system of nomenclature by MacKay (1959) is used, with the setae of the head named according to Heinrich (1916), those of the thorax and abdomen according to Hinton (1946) and those of the anal shield according to MacKay (1959). Voucher specimens were deposited in the museum of the Department of Entomology, University of Stellenbosch.

3.4 Results and Discussion

3.4.1 *Epichoristodes acerbella* (Fig. 3.1, Plate 3.1)

3.4.1.1 General

Larva elongate, light green with darker green stripe on dorsal midline and often yellow green stripes at lateral midlines. Integument rugose. Head yellow brown. Pinacula conspicuous, lighter than body colour. Spinules long, slender, lighter than body colour, easily observed. Prothoracic and anal shields yellow, easily observed. Prothoracic shield with dark pigmentation at lateral margins.

3.4.1.2 Head (Fig. 2.3 a-e; Plate 3.1 a,c)

Average width prior to pupation, based on 16 specimens, 1.04 mm. Vertical angle acute. Adfrontals narrow, extending to vertical angle. P^1 closer to Adf^2 than F^1. Ocellar areas rounded. Stemmata inconspicuous with exception of stemma III, surrounded by dark pigmentation. Stemma II closer to stemma III than stemma I. Stemma II separated from stemma I by distance greater than its diameter. Stemma III, IV and V in straight line, stemma VI at right angles to this line. O^1 equidistant from stemma II

and III. Line drawn through O^1 and A^2 closer to stemma II than I. Mandible with five teeth, second and third large and pointed, fourth smaller and slightly flattened, fifth straight-edged.

3.4.1.3 Thorax (Fig. 2.3 f,j; Plate 3.1 b,d)

On prothorax, spiracle small, circular, L1 equidistant from and in straight line with L2 and L3, SD1 equidistant from XD2 and SD2. On meso- and metathorax D1 dorsal to D2. Thoracic claws curved.

3.4.1.4 Abdomen (Fig 2.3 g-j)

Spiracles circular, smaller than setal bases. SD1 separated from spiracle by approximately half the diameter of spiracle. Spiracle on segment 8 (A8) on midventral line drawn through segment. L2 usually posteroventral to L1. SV group on A1, 2, 7, 8 and 9 usually 3:3:3:2:2.

Anal shield. Rounded and strongly tapered posteriorly. D1s further apart than L1s. D2s slightly shorter than L1s. D1s closer to corresponding SD1s than to each other. D1s anterior to SD1s.

Anal fork. Well developed, transparent, with 6-9 bluntly dentate prongs of approximately similar length. Width of base approximately equal or less length of prongs. Basal part of each prong tapered dorsally.

Prolegs. Prolegs with 32-54 and anal prolegs with 28-36 unevenly uniordinal, almost biordinal, crochets.

3.4.2 Comparison of final instar larvae of *E. acerbella*, *C. pomonella*, *G. molesta* and *T. leucotreta*

The larvae of *E. acerbella*, *C. pomonella*, *G. molesta* and *T. leucotreta* are morphologically close, all displaying the characteristic structure of tortricoid larvae: three setae in the lateral group on the prothorax, L1 and L2 always adjacent on A1-8, on segment nine D1 equidistant from D2 and SD1, but, if not, then in a straight line with them, SD1 on A8 usually anterior, anterodorsal or anteroventral to the spiracle, crotchets uniserial and circular or ovoid *in situ*, anal fork, if present, with straight prongs (MacKay 1959, Stehr 1987, Horak & Brown 1991, Komai 1999). Despite these similarities, characters for distinguishing between the different larvae are apparent, particularly between *E. acerbella*, a member of the subfamily Tortricinae and the other three species, which belong to the Olethreutinae. The most reliable taxonomic characters were the presence and structure of the anal comb and the structure and number of crotchets. Based on these characters, a key was developed to distinguish between final instar larvae of the different tortricid species feeding on deciduous fruit in South Africa.

3.4.2.1 Key to species

1. Anal comb absent, crochets unevenly uniordinal, 30 or 35 on prolegs and 15 or 25 on anal prolegs.……………………………………………………………………………...*C. pomonella*
   Anal comb present………………………………………………………………………………2
2. Anal comb transparent with well developed base, width similar to length of the prongs; crochets mostly uniordinal, 32-54 on prolegs and 28-36 on anal prolegs………………………..*E. acerbella*
   Anal comb dark, width of base equal to ¼ height of prongs ……….……….………...……….3
3. Crochets unevenly triordinal, reduced in medial half of anal prolegs, 36-42 on prolegs and 24-32 on anal prolegs…………………………………………..…………………………*T. leucotreta*
   Crochets unevenly uniordinal, 30 or 40 on prolegs, 25 on anal prolegs……..…………..*G. molesta*

3.5 References

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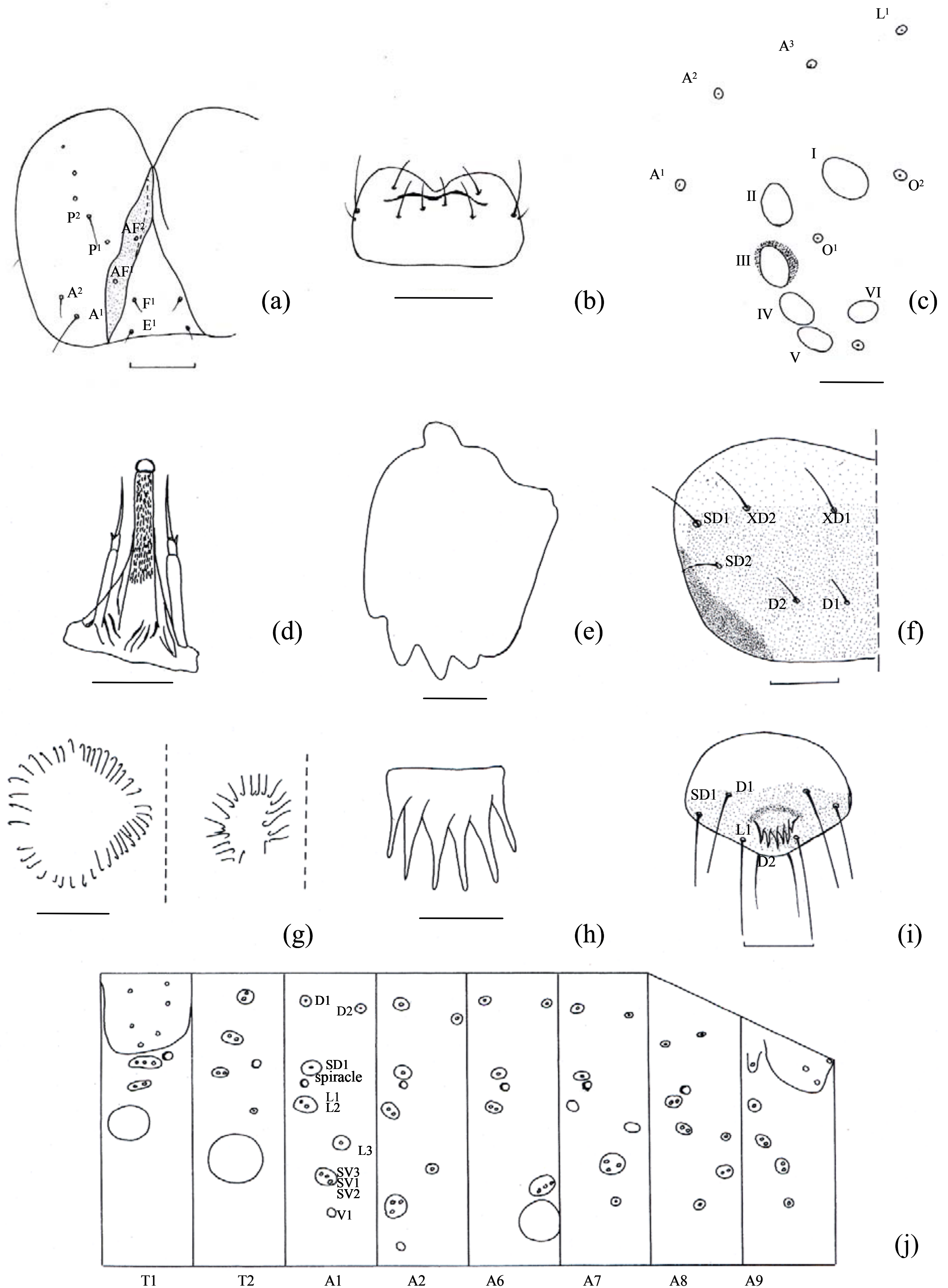
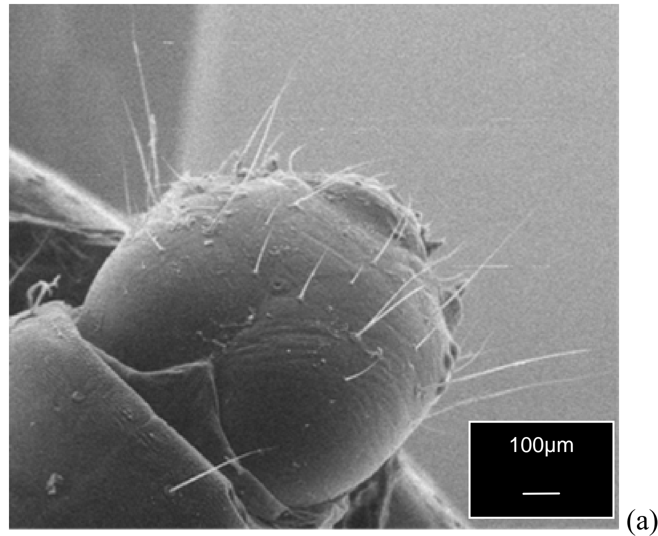
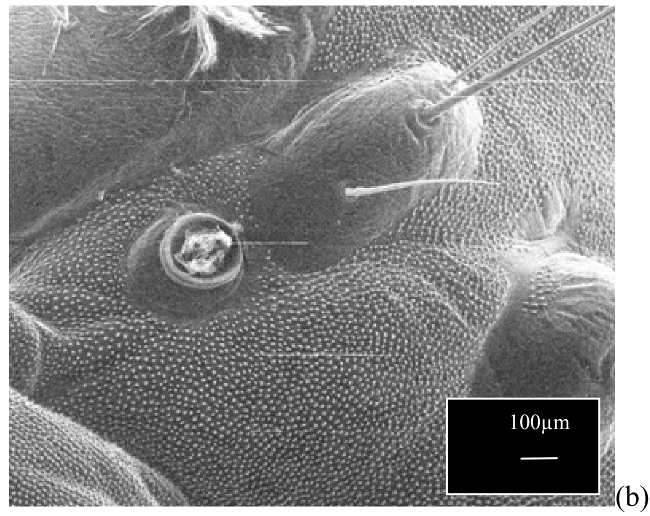


Fig. 3.1. *Epichoristodes acerbella* final instar larva. **a**, head (frontal aspect); **b**, labrum; **c**, stemmata; **d**, spinneret; **e**, mandible; **f**, prothoracic shield; **g**, crochets on prolegs and anal prolegs; **h**, anal comb; **i**, anal shield; **j**, setal map. Scale bar: a, f, i = 1mm, b-e, g-h = 0.1mm.

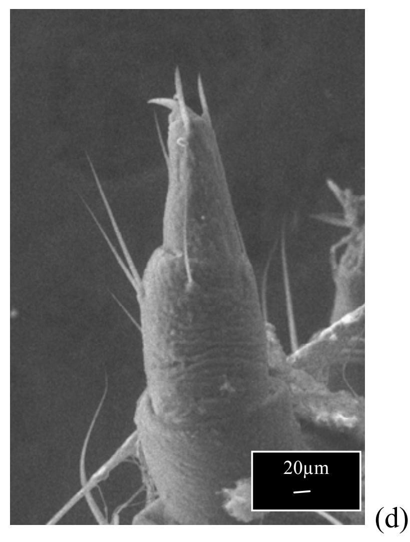


(a)



(b)

(c)



(d)

Plate 3.1. *Epichoristodes acerbella* final instar larva. **a**, head (dorsal aspect); **b**, spiracle on prothorax; **c**, antenna; **d**, prothoracic leg.

CHAPTER 4

Comparison of the pupal morphology of three tortricid (Lepidoptera) species of economic importance on tropical and subtropical fruit tree crops in South Africa

4.1 Abstract

The morphology of the pupae of the false codling moth *Thaumatotibia leucotreta*, the macadamia nut borer *T. batrachopa* and the litchi moth *Cryptophlebia peltastica* is described and illustrated. Keys are provided to distinguish between the pupae of the three species.

4.2 Introduction

Accurate identification of all stages of insect species of economic importance in agricultural ecosystems is essential for improved control and phytosanitary purposes. The adult and larval morphology of the economically important tortricid species on tropical and subtropical crops in South Africa, namely *Thaumatotibia leucotreta* (Meyrick), *T. batrachopa* (Meyrick) and *Cryptophlebia peltastica* (Meyrick), has been described (Meyrick 1908, Meyrick 1921, Stofberg 1948, Bradley 1953, Swart 1966, Krüger 1998, Chapter 2). However, descriptions of the pupal stages of these species are lacking. Therefore, the primary aim of this study was to compare the morphology of the pupae of these three species and to develop keys to distinguish between them.

4.3 Material and methods

4.3.1 Insect material

Insect material of *T. leucotreta* was obtained by donation from an established laboratory colony (Ceder Biocontrol, Citrusdal) and that of *T. batrachopa* by field collection (Institute for Tropical & Subtropical Crops, Nelspruit, 2003). A laboratory colony of *C. peltastica* was established in the Department of Entomology at the University of Stellenbosch from material collected from the Institute for Tropical &

Subtropical Crops, Nelspruit during 2003 to obtain material for analysis. Voucher specimens were deposited in the museum of the Department of Entomology, University of Stellenbosch.

4.3.2 Light microscopy

Digital photomicrographs were taken using a Wild M8 stereo microscope with a 5 mega pixel Nikon Coolpix camera and used to generate line drawings. Measurements were taken using an ocular micrometer.

4.3.3 Nomenclature

Nomenclature follows that of Mosher (1916) with modifications by Adler (1991).

4.4 Results

Pupae of *T. leucotreta*, *T. batrachopa* and *C. peltastica* display the general characteristics of tortricoid pupae: antenna extending almost to wing tip; maxillae two-fifths the lengths of the wings, labial palpi half the length of the maxillae; wings broad but never pointed, extending to posterior of abdominal segment three (A3); male pupae with four independently moveable abdominal segments, female pupae with three; A4-7 bearing two rows of dorsal spines (Mosher 1916, Adler 1991). All three species also display the characteristics of pupae of the Eucosmini: A8–A10 with one row of spines, cremaster reduced or absent, maxillae 1.4-2.1 times as long as labial palpi, cauda with four to eight flat, hooked setae, anal rise with two hooked setae on either side (Evenhuis *et. al* 1973, Zimmerman 1978, Patočka 1980, Horak & Brown 1991). Additional characters are described below for each of the aforementioned species.

4.4.1 *Thaumatotibia leucotreta* (Fig. 4.1)

4.4.1.1 General

Pupa 7.94-9.80 mm in length, based on 16 specimens, brown, frontal region dark brown. Pupa formed without cocoon in fruit or enclosed in a cocoon formed from soil particles and debris in soil. Distinct cremaster absent.

4.4.1.2 Head

Front with slight, broad mesal ridge, bearing one pair of setae. Eyes large, prominent. Maxilla length along midline about 1.5 times length of labial palpus. Structure of antenna with signs of sexual dimorphism: thickened, prominent in males relative to females; extending beneath the mesocoxa by a length approximately equal to the mesocoxa along the midline in females, in males by a length greater than that of the mesocoxa, almost to the tip of the mesotarsus in males.

4.4.1.3 Thorax

Segments well delimited dorsally. Forewing almost completely obscuring hindwing ventrally. Protarsus extending beyond procoxa by approximately 1/4 length of mesocoxa. Metatarsus well developed. Coxa of metathorax slightly visible in female, more exposed in male.

4.4.1.4 Abdomen

Spiracles oblong, prominent. A2-3 lacking dorsal cavities and crossfolds. A4-7 with posteriorly directed dorsal spines, anterior row distinct, posterior row indistinct, with 3-7 dorsal spines between anteromesad setae. A8-10 with a single row of prominent posteriorly directed dorsal spines. Anal rise with thickened, distinctly curled setae situated anteriorly to anal opening and to the outermost ventral spine on the ninth segment. Sexual dimorphism distinct. Females with genital openings ventromedially on anterior margins on segment nine and extending to anterior margin of segment ten; anterior pair of setae on anal rise usually adjacent to anal opening. Males with genital aperture ventromedially on segment nine, anterior pair of setae on anal rise anterior to anal pore.

4.4.2 *Thaumatotibia batrachopa* (Fig. 4.2)

4.4.2.1 General

Pupa 6.14-8.29 mm in length, based on 8 specimens, dark brown; found in the ground encased in cocoon constructed of soil particles, cocoon absent if pupation takes place directly in fruit. Distinct cremaster absent.

4.4.2.2 Head

Front with slight, broad mesal ridge, bearing one pair of setae. Eyes prominent, large. Antenna extending below mesocoxa by length approximately equal to mesocoxa in females. Maxilla length along midline approximately equal to twice length of labial palpus.

4.4.2.3 Thorax

Segments well delimited dorsally. Forewing almost completely obscuring hindwing ventrally. Metatarsus clearly delimited.

4.4.2.4 Abdomen

Spiracles oblong, inconspicuous. A2-3 lacking dorsal cavities and crossfolds. A4-7 with posteriorly directed dorsal spines, anterior row conspicuous, posterior row inconspicuous, bearing 4-8 dorsal spines between anteromesad setae. A8-10 with single row of prominent, posteriorly directed dorsal spines. Sexual dimorphism of pupa distinct. Female pupa with broad genital opening ventromedially on anterior margins on segment nine, posteriorly lined with flattish white setae; anal rise with two pairs of thickened, distinctly curled setae, anterior pair situated just anterior to anal pore opening. Male pupa with genital aperture ventromedially on ninth segment, setae on anal rise situated adjacent to anal pore, anterior pair may occur slightly anterior to the opening of the anal pore.

4.4.3 *Cryptophlebia peltastica* (Fig. 4.3)

4.4.3.1 General

Pupa dark brown, with darker patches on the pronotum; frontal region dark brown; 8.59–10.63 mm in length, based on 10 specimens. Cocoon of soil particles and debris present if pupation takes place in soil, absent if pupation occurs in fruit. Distinct cremaster absent.

4.4.3.2 Head

Front with slight, broad mesal ridge, bearing one pair of setae. Eyes large, prominent. Antenna prominent, extending beyond mesocoxa by length less than that of mesocoxa. Length of maxilla along midline 1.5 times that of labial palpus.

4.4.3.3 Thorax

Segments well delimited dorsally. Pronotum more than four times as long as vertex along midline. Forewing almost completely obscuring hindwing ventrally. Protarsus extending beyond procoxa by approximately 1/5 length of mesocoxa. Metatarsus well developed, clearly delimited. Coxa of metathorax slightly exposed.

4.4.3.4 Abdomen

Spiracles oblong, indistinct. A2-3 lacking dorsal cavities and crossfolds. A4-7 with posteriorly directed dorsal spines, anterior row prominent, posterior row inconspicuous, bearing 4–12 dorsal spines between anteromesad setae. A8-10 with single row of prominent, posteriorly directed dorsal spines. Anal rise with two pairs of thickened, distinctly curled setae medially adjacent to the ventral spines on A10; both pairs of setae situated anterior to anal pore opening in both males and females. Sexual dimorphism of pupae distinct. Female pupa with genital openings ventromedially on anterior margins on A9 and extending to the anterior margin of A10. Male pupa with genital aperture ventromedially on A9.

4.5 Discussion

In the Lepidoptera, the structure of the cremaster often provides structural features useful for identifying pupae of different species. The absence of a distinct cremaster in *T. leucotreta*, *T. batrachopa* and *C. peltastica* necessitated the search for other characters for distinguishing between the species. Upon examination of the pupae of the three species numerous morphological similarities were noted. However, several structural differences were also observed. These differences were used to construct a key to distinguish between the pupae of the three species.

4.5.1 Key to species

1. Anal rise with setae adjacent to ventral spine on A10; both pairs of setae situated anterior to anal pore opening; A4-7 with 4–12 dorsal spines between anteromesad setae; protarsus extending beyond procoxa by approximately 1/5 length of mesocoxa…….…………….....……...*C. peltastica*

   Anal rise with setae not adjacent to ventral spine on A10….….…………………………….……...2

2. Protarsus extending beyond procoxa by approximately 1/4 length of mesocoxa; A4-7 with 3–7 dorsal spines between anteromesad setae; females with setae on anal rise adjacent to anal pore, males with anterior pair of setae on anal rise anterior to anal pore…………………….….…*T. leucotreta*

Protarsus extending beyond procoxa by approximately 1/5 length of mesocoxa; A4-7 with 4–8 dorsal spines between anteromesad setae; females with anterior setae on anal rise anterior to anal pore opening, males with both pairs of setae usually adjacent to anal pore ……….…………………………………………………………………………..…*T. batrachopa*

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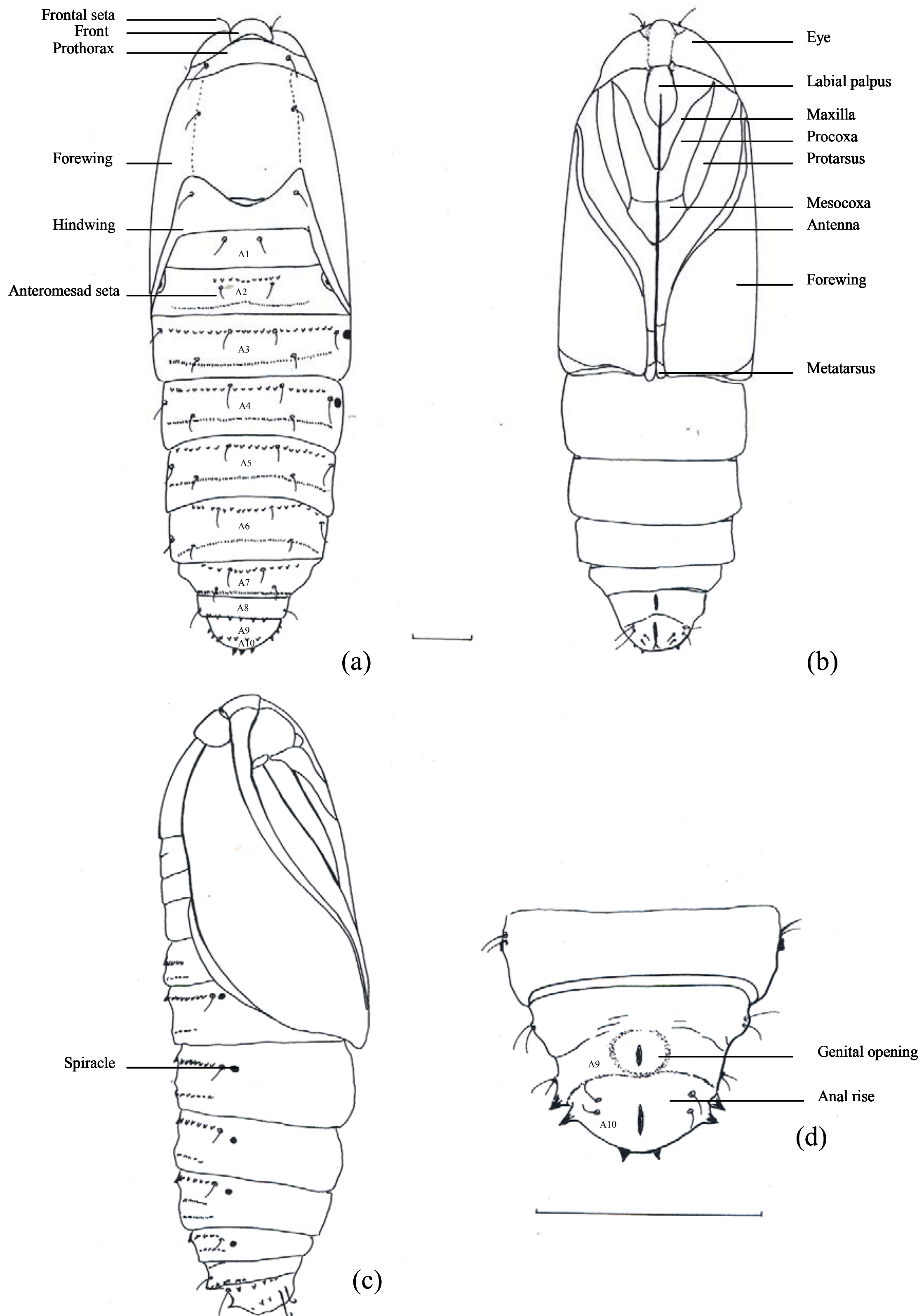

Fig. 4.1. *Thaumatotibia leucotreta* pupa. **a**, dorsal aspect (female); **b**, ventral aspect (female); **c**, lateral aspect (female); **d**, terminal abdominal segments (male). Only prominent setae are shown. Scale bar = 1mm.

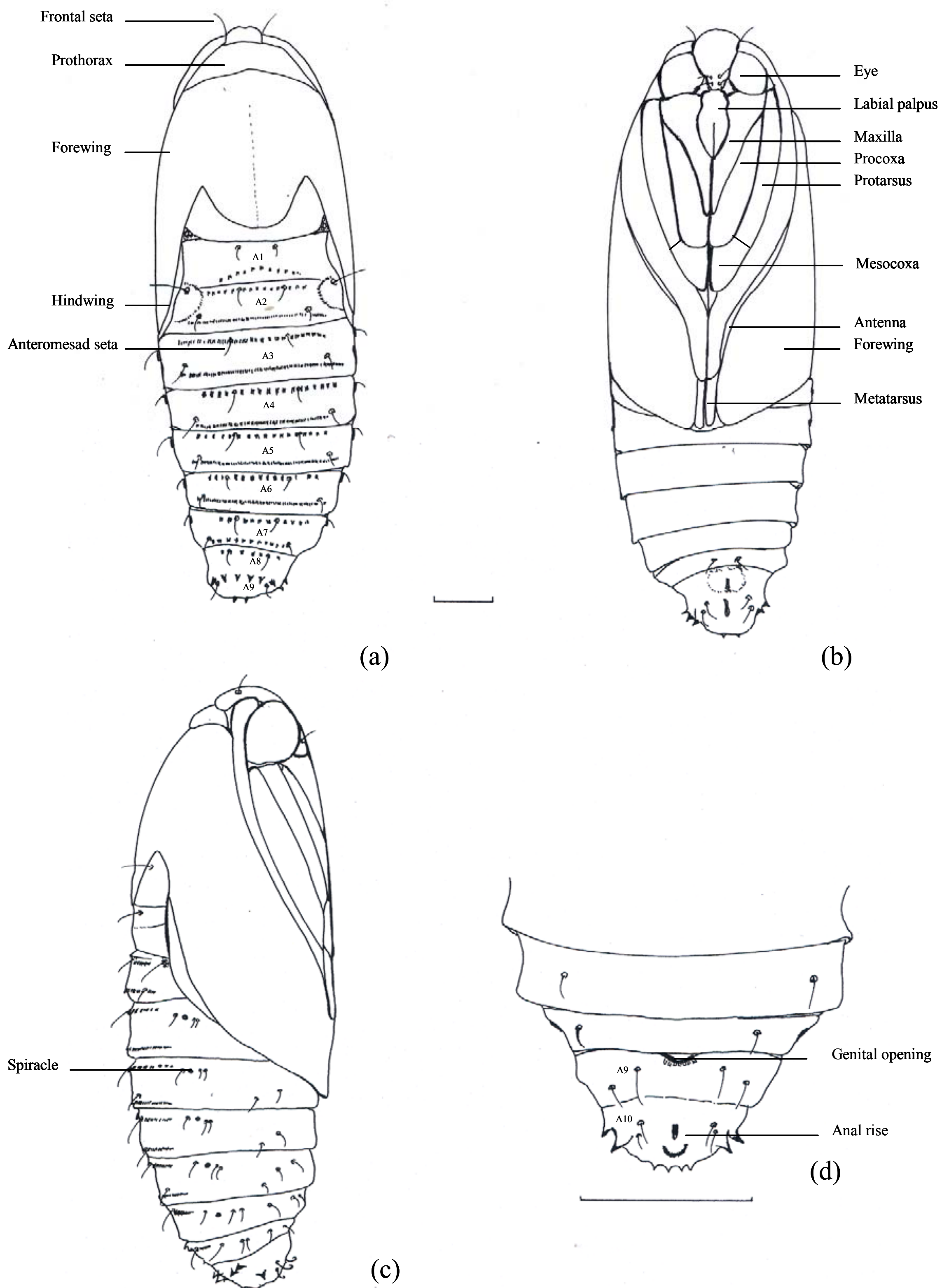

Fig. 4.2. *Thaumatotibia batrachopa* pupa. **a**, dorsal aspect (male); **b**, ventral aspect (male); **c**, lateral aspect (male); **d**, terminal abdominal segments (female). Only prominent setae are shown. Scale bar = 1mm.

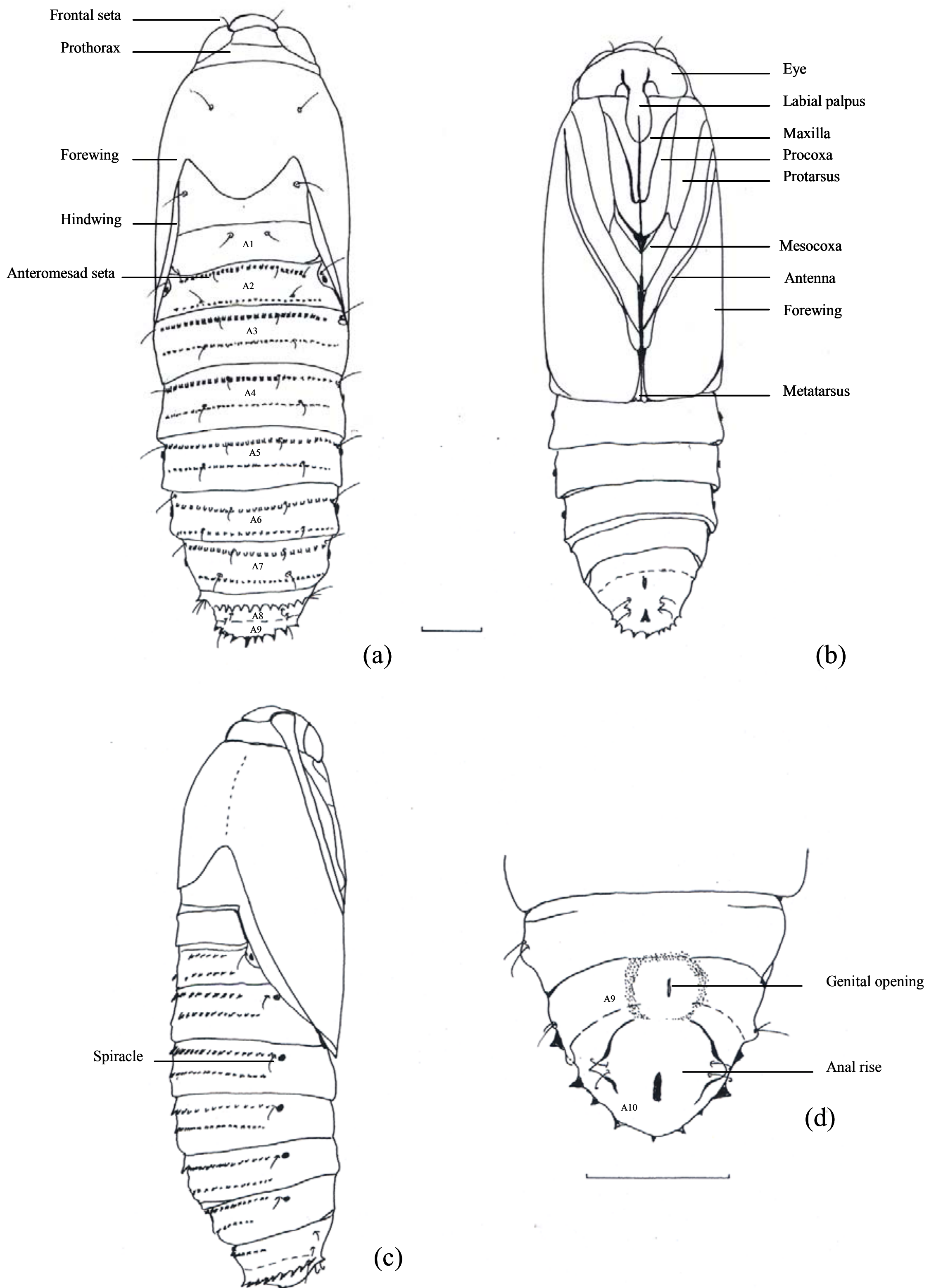

Fig. 4.3. *Cryptophlebia peltastica* pupa. **a**, dorsal aspect (female); **b**, ventral aspect (female); **c**, lateral aspect (female); **d**, terminal abdominal segments (male). Only prominent setae are shown. Scale bar = 1mm.

CHAPTER 5

Description of the pupa of *Epichoristodes acerbella* (Lepidoptera: Tortricidae) and comparison with pupae of other tortricid species of economic importance on deciduous fruit tree crops in South Africa

5.1 Abstract

The morphology of the pupa of the pear leafroller *Epichoristodes acerbella* is described, illustrated and compared with the pupae of other tortricids of major economic importance to the deciduous fruit tree crop industry in South Africa, namely codling moth *Cydia pomonella*, oriental fruit moth *Grapholita molesta* and the false codling moth *Thaumatotibia leucotreta*. Keys are provided to distinguish between the pupae of the four species.

5.2 Introduction

Four Tortricidae (Lepidoptera) are of major economic importance to the South African deciduous fruit tree crop industry. They are the codling moth *Cydia pomonella* (L.), the oriental fruit moth *Grapholita molesta* (Busck), the false codling moth *Thaumatotibia leucotreta* (Meyrick) and the pear leafroller *Epichoristodes acerbella* (Walker). Distinguishing between all the life stages of these species, particularly the immature stages, is of importance for both effective control and phytosanitary purposes. The larvae of all four species have been described (Stofberg 1948, MacKay 1959, Chapters 2 & 3) as well as the pupae of *C. pomonella* (Mosher 1916, Adler 1991), *G. molesta* (Adler 1991, Komai 1999) and *T. leucotreta* (Chapter 4). However, a description of the pupa of *E. acerbella* is lacking. Therefore, the aims of this study were to (1) describe, with illustrations, the morphology of the pupa of *E. acerbella*, (2) compare the pupal morphology of *C. pomonella*, *G. molesta*, *T. leucotreta* and *E. acerbella* and (3) develop keys for distinguishing between the pupae of the four species.

5.3 Material and methods

Established laboratory colonies provided material for analysis of *C. pomonella* (Hortec, Stellenbosch), *G. molesta* (Agriculture and Agri-Food, Canada), *T. leucotreta* (Ceder Biocontrol, Citrusdal) and *E. acerbella*

(Department of Entomology, University of Stellenbosch). Voucher material was deposited in the museum of the Department of Entomology, University of Stellenbosch. Digital photomicrographs were taken using a Wild M8 stereo microscope with a 5 mega pixel Nikon Coolpix camera and used to generate line drawings. Measurements were taken using an ocular micrometer.

5.4 Results and discussion

5.4.1 Description of *E. acerbella* pupa (Fig. 5.1)

5.4.1.1 General

Pupa uniformly yellow brown or with abdominal segments darker ventrally, 9.53-12.50 mm in length, based on 16 specimens.

5.4.1.2 Head

Front smoothly rounded or with slight, broad mesal ridge, bearing one pair of setae. Maxilla along midline approximately 2.0-2.5 times length of labial palpus. Antenna extending below mesocoxa by a length greater than the length of the mesocoxa, thickened in males relative to females.

5.4.1.3 Thorax

Segments well delimited dorsally. Pronotum along midline more than four times as long as vertex. Forewing almost completely obscuring hindwing ventrally. Protarsus extending beyond procoxa by approximately 4/5 length of mesocoxa. Metatarsus well developed, clearly delimited. Coxa of metathorax exposed.

5.4.1.4 Abdomen

Spiracles prominent, oblong. Segments 2-3 (A2-3) lacking dorsal cavities and crossfolds. A2 with anterior and posterior rows of dorsal spines slight. A3-7 with anterior row of dorsal spines readily apparent and posterior row slight. A8 with dorsal spines virtually absent. A4-7 with 5–12 spines between anteromesad setae. Cremaster present, well developed, elongate, length greater than width, tip broadly flattened. Four pairs of thickened, distinctly curled cremastral setae.

5.4.2 Comparison of the pupae of *E. acerbella, C. pomonella, G. molesta and T. leucotreta*

The diagnostic characters common to tortricoid pupae are displayed by *C. pomonella*, *G. molesta*, *T. leucotreta* and *E. acerbella*: antenna extending almost to wing tip; wings broad but never pointed, extending to posterior of abdominal segment three; male pupae with four, female pupae with three independently moveable abdominal segments; A4-7 bearing two rows of dorsal spines and maxillae well developed (Mosher 1916, Adler 1991, Horak & Brown 1991). However, considerable morphological variation can be found between the pupae of the four species, with *C. pomonella*, *G. molesta* and *T. leucotreta* appearing closer to each other than *E. acerbella*. This may reflect the relationships of the species since *C. pomonella*, *G. molesta* and *T. leucotreta* are members of the subfamily Olethreutinae whereas *E. acerbella* belongs to the Tortriciniae (Horak & Brown 2005). Pupae of *C. pomonella*, *G. molesta* and *T. leucotreta* have the following characters in common with members of the closely related tribes Grapholitini (*C. pomonella* and *G. molesta*) and Eucosmini (*T. leucotreta*): A8 with one to two rows of dorsal spines, A9 and A10 usually with one row of dorsal spines; maxillae 1.4-2.1 times as long as labial palpi; cremaster reduced or absent, anal rise with two hooked setae on either side (Mosher 1916, Zimmerman 1978, Adler 1991, Horak & Brown 1991). In contrast, *E. acerbella,* a member of the subfamily Tortricinae, tribe Archipini, displays the pupal characters typical for this group: A8 with one or two rows of spines, A9 only rarely with a well developed row of spines, cremaster well-developed, often cylindrical and longer than wide, with eight strong hooked setae. Based on the aforementioned characters and others, a key was constructed to distinguish between the pupae of *C. pomonella, G. molesta, T. leucotreta* and *E. acerbella*.

5.4.2.1 Key to species

1. Cremaster present, well developed with eight thickened, hooked setae, A9 only rarely with well developed row of spines, maxilla approximately 2.0-2.5 times length of labial palpus ………………………………...………………………………..….……..…..*E. acerbella*
   Cremaster absent………………………………………………………….……………………2
2. Spiracles circular; A4-6 with single anterior row of dorsal spines; terminal spines extending anteroventral to lateral-most terminal setae…………………….………………………………*G. molesta*
   Spiracles oblong…………………………………………………………….…………………3
3. Length of setae on anal rise equal to distance between them; spines on A9 prominent on anterior margin; medial line of labial palpi extending halfway towards interior…………..……*T. leucotreta*

Length of setae on anal rise longer than distance between them; spines on A9 small; medial line of labial palpus extending three-quarters towards interior……………………………..*C. pomonella*

5.5 References

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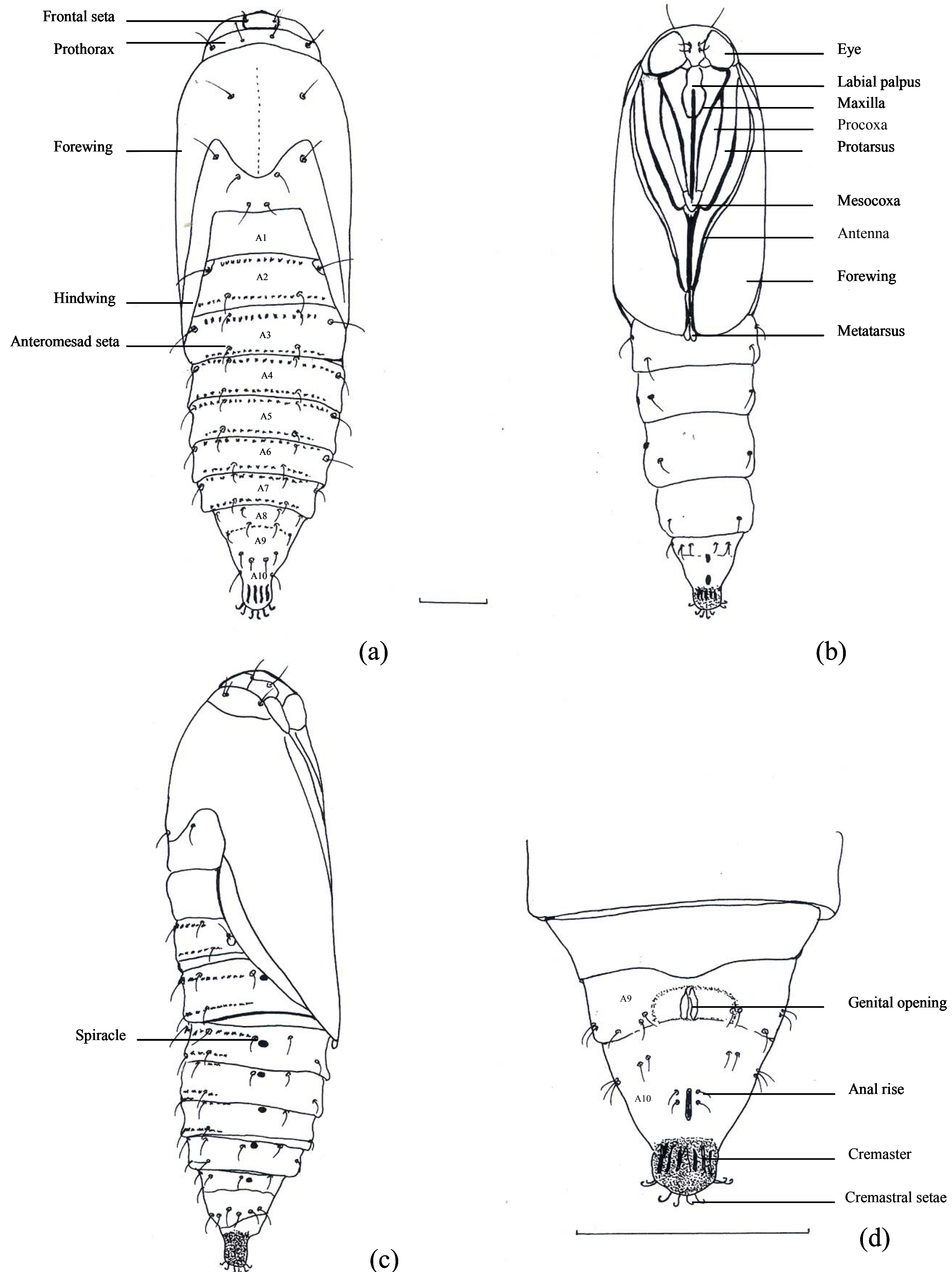

Fig. 5.1. *Epichoristodes acerbella* pupa. **a**, dorsal aspect (female); **b**, ventral aspect (female); **c**, lateral aspect (female); **d**, terminal abdominal segments (male). Only prominent setae are drawn. Scale bar = 1mm.

CHAPTER 6

Population genetic structure of the oriental fruit moth *Grapholita molesta* (Lepidoptera: Tortricidae) in South Africa, inferred by AFLP analysis

6.1 Abstract

The population genetic structure of the oriental fruit moth, *Grapholita molesta*, the key pest of stone fruit worldwide, was characterized in the major stone-fruit growing regions in South Africa. The amount and distribution of variation in populations was determined in order to gain insight into gene flow over various spatial scales. Populations were collected from six geographically distant regions and compared with each other as well as with a population obtained from Canada. Amplified fragment length polymorphism (AFLP) with five selective primer pairs was used to generate 250 fragments for analysis. The South African and Canadian populations could be clearly distinguished and a significant amount of population differentiation was found within the South African population ($G_{st} = 0.279$). It was possible to distinguish among populations collected from orchards situated within one kilometre of each other. Populations from different areas clustered into two main groups, but no significant correlation between genetic and geographic distance was found. It is suggested that *G. molesta* was introduced at least twice into South Africa and that the movement of fruit, bins and nursery material between areas may also have played an important role in extending the range of this insect. These results provide important information on the population genetics of *G. molesta* for the design and implementation of sustainable pest management strategies.

6.2 Introduction

The oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), is one of the most destructive pests of stone fruit worldwide and the key pest in South African stone fruit orchards. *G. molesta* larvae cause indirect damage by feeding on twigs, causing shoot dieback. Damage caused by *G. molesta* in South African peach orchards has been considerable and the insect has posed a threat to the future of the canning peach industry (Blomefield & Barnes 2000). *G. molesta* was first described from

South Africa in 1990, but may have been present earlier (Blomefield & Geertsema 1990). It was speculated that the species was most likely introduced into the country on peach budwood imported illegally (Blomefield & Geertsema 1990, Blomefield *et al*. 1990). The moth has since been observed in all stone fruit growing regions in South Africa. Currently, management of *G. molesta* population levels in South Africa is based on the use of the insecticide azinphos-methyl as well as pheromone-based mating disruption (Blomefield 1996, Blomefield & Barnes 2000).

When designing and implementing pest management programs, the dispersal capability of the insect pest is an important factor to consider. The flight range of *G. molesta* using mark-recapture techniques has been estimated several times in Canada and in the USA. These studies found that *G. molesta* dispersal was limited. The majority of adults did not disperse over distances greater than 200 m, leading to localized infestations occurring in the same spots within orchards over successive seasons (Summers 1966, Sziraki 1979, Vickers *et al*. 1985). It was therefore suggested that migration would only play a role in the dispersal of the species when considered within an in-orchard basis (Rothschild & Vickers 1991). However, flight distances have been recorded for a few individuals ranging from one to nearly two miles (Yetter 1932, Steiner & Yetter 1933, Sziraki 1979). It is not known whether these estimates of dispersal can be generalized to oriental fruit moth populations across the world.

Dispersal estimates of insect species are increasingly being calculated using molecular information, which complements estimates based on traditional methods. Molecular studies are able to take into account factors other than flight distance at a given time. They are able to provide insight into historic patterns of movement as well as provide long-time estimates that are integrated over the geographic range of the insect over many generations (Roderick 1996, Bohonak 1999). Therefore, studies determining the population genetic structure of insect pests have become of primary concern since they are able to make a valuable contribution to the understanding of pest dispersal and subsequently also management practices.

A number of molecular markers have been used for determining the population genetic structure of lepidopteran pests. Amplified fragment length polymorphism (AFLP) analysis (Vos *et al*., 1995) is one of the most recently described that has proved useful for analysis of species such as the gypsy moth *Lymantria dispar* (Reineke *et al*. 1999), the fall armyworm *Spodoptera frugiperda* (McMichael & Prowell 1999) and the winter pine processionary moth *Thaumetopoea pityocampa – wilkonsoni* complex (Salvato *et al*. 2002). The limitations of AFLP analysis, namely that fragments must usually be treated as dominant loci and no information can be obtained about the gene loci, do not outweigh the advantages of AFLP analysis being highly reproducible, requiring only minimal development time and generating a large number of fragments for analysis.

The aims of this study were therefore twofold, namely to determine the population genetic structure (amount and distribution of genetic variation) of *G. molesta* populations in South Africa using AFLP analysis over both large and local geographic scales and secondly to use this information to draw conclusions about *G. molesta* dispersal by comparison with dispersal estimates based on mark-recapture techniques.

6.3 Material and methods

6.3.1 Insect material

Grapholita molesta samples were collected from peach orchards from six geographically distant regions in the Western Cape Province, South Africa (Table 6.1, Fig. 6.1) during January 2004 (Stellenbosch) and November 2004-February 2005 (Caledon, Ceres, Elgin, McGregor and Tulbagh). To gain a measure of intrapopulation variability over smaller geographic scales, samples were collected from orchards situated within one kilometre of each other from Tulbagh and Stellenbosch. For comparison with the South African population, samples of foreign origin were obtained from a laboratory colony maintained in Canada (Agriculture and Agri-Food, Ontario). A total of 92 specimens were included for analysis. Voucher material of each specimen included for analysis was deposited in the museum of the Department of Entomology, University of Stellenbosch.

6.3.2 AFLP analysis

Genomic DNA was extracted from the heads and legs of moths using the CTAB protocol described by Reineke *et al*. (1998) with reduced volumes. AFLP analysis was conducted using standard procedures (Vos *et al*. 1995) with minor modifications as previously described (Timm *et al.* 2005). Genomic DNA (200 ng) was digested with five units each of the restriction enzymes *Eco*RI (Promega) and *Mse*I (New England Biolabs) for three hours at 37°C in One-Phor-All buffer (Pharmacia) and 0.1 µg/µl BSA (New England Biolabs). To each of these reactions, 5 pmoles of *Eco*RI adaptor, 50 pmoles of *Mse*I adaptor, 1 unit of T4 DNA ligase (Promega) and 1 mM ATP (Roche) was added and incubated overnight at 37°C. These reactions were diluted 1:10 in 1 x TE buffer and 13 µl used for preselective amplification.

Preselective amplification reactions consisted of 75 ng *Eco*RI primer (5'- GAC TGC GTA CCA ATT C-3') and *Mse*I primer (5'- GAT GAG TCC TGA GTA A-3'), 200 µM of all four dNTPs, 1 unit of *Taq*

polymerase (Promega) and 1.5 mM $MgCl_2$. Amplification was performed in the GeneAmp PCR Instrument System 2720 DNA thermocycler (Applied Biosystems) using the following cycle program: 5 minutes at 72°C followed by 30 cycles with the cycle profile 30 seconds at 94°C, 1 minute at 56°C and 1 minute at 72°C. The reaction was completed with a final extension period of 72°C for 5 minutes and stored at 4°C. Amplification products were diluted tenfold in 1 x TE buffer and used as templates for selective amplification.

Selective amplification was carried out using five selective primer combinations, after an initial 25 combinations were screened, with each primer including three selective nucleotides (*Eco*RI-GAC *Mse*I-GCA, *Eco*RI-CTG *Mse*I-CAT, *Eco*RI-AGT *Mse*I-CTA, *Eco*RI-ATC *Mse*I-CAC, *Eco*RI-ATC, *Mse*I-CAA). *Eco*RI selective primers were end-labelled prior to amplification using 5 ng *Eco*RI selective primer, 0.25 µCi [γ-^{33}P] ATP (Easytides, NEN) and 0.05 units T4 polynucleotide kinase (Promega) in One-Phor-All buffer and incubating the reaction for two hours at 37 °C. Selective amplification was carried out using 2.5 µl diluted pre-amplified DNA, 200 µM of each dNTP, 0.025 units Taq (Promega), 1.5 mM $MgCl_2$, 15 ng *Mse*I primer and 0.5 µl labeled *Eco*RI primer. The cycle profile was as follows: 30 seconds at 94 °C, 30 seconds at 65 °C, and 1 minute at 72 °C. The annealing temperature was reduced by 0.7 °C for the next 12 cycles and continued at 56 °C for 24 cycles.

Selective amplification products were electrophoresed on 6% (w/v) denaturing polyacrylamide gels at 60 W for 2-3 hours. Gels were dried on Whatmann paper and exposed to Kodak Biomax X-ray films for visualization.

6.3.3 Data analysis

AFLP products were scored qualitatively as the presence (1) or absence (0) of fragments to create a binary matrix that was used in subsequent calculations. As a measure of population diversity, Nei's estimate of gene diversity (*H*) (Nei 1987) was derived using POPGENE version 1.31 (Yeh & Yang 1997). Genetic diversity was also calculated as the percentage polymorphic fragments (95% criterion) present in populations. Additional measures of population diversity included the proportion of rare fragments, defined as fragments that occurred in 10% or fewer individuals, as well as the number of private fragments, namely fragments that were unique to a population or region.

Population differentiation was investigated using estimates of G_{st}, obtained using POPGENE version 1.31 (Yeh & Yang 1997). These estimates, in turn, were used to calculate the average number of individuals that migrate to each population per generation using the formula $Nm = \frac{1}{4}[(1/G_{st}) - 1]$ (Wright 1951).

To investigate the relationships among populations, pair-wise measures of genetic identity (*I*) and genetic distance (*D*) were calculated using Nei's (1978) unbiased genetic distance algorithm. These measures of genetic distance were used to construct a dendrogram using the unweighted pair group method with arithmetic mean (UPGMA) metric in POPGENE version 1.31 (Yeh & Yang 1997). Relationships among individuals and populations were also viewed using Q-mode principal coordinate analysis (PCOA), performed using MVSP Version 3.11c (Kovach 1999) and based on a dissimilarity matrix generated using Gower's general similarity co-efficient (Sneath & Sokal 1963).

The distribution of genetic variability within and among populations was investigated by an analysis of molecular variance (AMOVA) using the software GenAlEx version 6 (Peakall & Smouse 2005), with the number of permutations set at 1000. This software was also used to test the association between genetic distance and geographic distance using a Mantel test (Mantel 1967).

6.4 Results

6.4.1 AFLP banding patterns

From the combined AFLP banding patterns for the five selective primer pairs, a total of 236 fragments were scored for analysis in the South African *G. molesta* population. In addition, 14 fragments were scored in the Canadian population that were not present in the South African population. Comparison of AFLP profiles showed a clear differentiation of *G. molesta* from the South African and Canadian populations.

6.4.2 Genetic diversity

The percentage of polymorphic loci (*p*) was 100% for the South African population and 90.94% for the mean of the populations. Estimates of genetic diversity among populations varied considerably, with *p* ranging from 99.43% in the Caledon population to 58.18% in the Ceres population and *H* from 0.186 in the Caledon population to 0.100 in the Ceres population (Table 6.2). These estimates of genetic diversity closely mirrored those obtained by comparison of the average number of rare fragments among populations, with the highest proportion of rare fragments being present in the Caledon population and the lowest in the Ceres population. All populations had rare fragments. The McGregor population had a

single private fragment, followed by the Elgin population with two, the Ceres population with four, the Tulbagh population with five, and the Stellenbosch population with seven. The highest number of private fragments was recorded in the Caledon population, which had 15.

6.4.3 Genetic differentiation

Estimated population differentiation in the South African population averaged over all loci, assuming a random mating model in Hardy-Weinberg equilibrium, was $G_{st} = 0.279$. The corresponding migration coefficient *Nm* i.e. the absolute number of individuals exchanged among populations per generation was calculated as 0.646.

6.4.3.1 Relationships among geographical populations

Estimates of genetic distance and genetic identity (Nei 1978) between pairs of populations were calculated, based on 250 fragments scored (Table 6.3). A genetic distance value of 0.1963 and genetic identity index of 0.8217 was calculated between the South African and Canadian populations (pooled data not shown). In the South African population genetic distance estimates ranged from 0.0160 (McGregor and Stellenbosch) to 0.1923 (Elgin and Ceres). Genetic identity indices ranged from 0.9841 (McGregor and Stellenbosch) to 0.8250 (Elgin and Ceres).

To represent the relationships among populations, cluster analysis (UPGMA) was used to generate a dendrogram based on genetic distance estimates (Fig. 6.2). Cluster analysis revealed that the South African and Canadian populations were clearly separated into two clusters. Within the South African population, a major division was apparent between the Ceres population and all other populations. From the UPGMA dendrogram it was apparent that patterns of population clustering were not related to geographic distance. This was confirmed using a Mantel test, which was conducted to establish the relationship between genetic and geographic distance. No significant correlation was found between the two matrices ($r = 0.0138$, $P = 0.256$).

6.4.3.2 Genetic variation over local geographic scales

The relationships among *G. molesta* populations over limited geographic scales was investigated by examining populations collected from orchards situated within one kilometre of each other from three farms in Tulbagh and Stellenbosch. These populations were found to be genetically differentiated and principal co-ordinate analysis could be used to distinguish among populations. However, discordant individuals were occasionally found (Fig. 6.3).

The distribution of variability within and among populations from closely-situated orchards was investigated using AMOVA analysis (Table 6.4). For all regions, a significantly high proportion of the variability could be attributed to variation within populations. Variation among orchards was also significant ($P < 0.001$) and varied from 15% (Tulbagh) to 20% (Stellenbosch).

6.5 Discussion

6.5.1 Molecular marker

This study has shown AFLP analysis to be effective in distinguishing among *G. molesta* populations situated within one kilometre of each other. The results produced and the high level of reproducibility obtained, indicate that AFLP analysis is a marker well suited for determining the population genetic structure of a tortricid species.

6.5.2 Genetic diversity

Estimates showed that a high level of genetic diversity was present in the South African *G. molesta* population (p = 100%, H = 0.182). The highest estimates of genetic diversity, based on various measures, were consistently found in the Caledon population. In contrast, genetic diversity estimates in the Ceres population were lower than those of all other populations as well as for the population means of the species in South Africa. All populations had private fragments, indicating that populations were genetically differentiated.

6.5.3 Genetic differentiation

A significant amount of genetic variation was found among geographic populations. The UPGMA cluster analysis revealed that populations were clearly separated into three clusters. The Canadian population formed a cluster distinct from the South African population and within the latter the Ceres population was clearly separated from the remaining populations. A genetic distance estimate of 0.1963 and a genetic identity index of 0.8217 was calculated between the South African and Canadian populations. The origin of *G. molesta* in South Africa is not known, although the species is indigenous to northwest China

(Roehrich 1961). The large amount of variation between the Canadian and South African populations may preclude the possibility of the South African *G. molesta* population originating from Canada, although a phylogenetic study would be necessary as confirmation. *Grapholita molesta* populations from Canada, however, show distinct biological differences to those from South Africa. For example, Canadian populations have three to four generations per season (Pree *et al*. 1994) whereas South African populations have between four and six (Blomefield & Barnes 2000).

Within the South African *G. molesta* population, differentiation was found to be significant (G_{st} = 0.2792, *Nm* = 0.6455). Cluster analysis clearly showed that the Ceres population was distinct from the remaining populations. Relatively large genetic distances were recorded between the Ceres population and all other South African populations, varying between 0.1095 (Ceres and Tulbagh) and 0.1923 (Ceres and Elgin). Indices of genetic identity between the Ceres population and all other South African populations sampled were much lower than those between the remaining populations, ranging between 0.8250 (Ceres and Elgin) and 0.8872 (Ceres and Tulbagh). It is unlikely that random genetic drift or selection could have accounted for the large amount of genetic variation observed between the Ceres and remaining South African populations. Climatic conditions do not differ vastly among the regions and control practices in all regions are similar, being limited to pheromone-based mating disruption as well as insecticide control using azinphos-methyl. The results found in this study therefore appear to suggest that, contrary to published information (Blomefield & Geertsema 1990), it is likely that *G. molesta* was introduced into South Africa more than once.

Grapholita molesta in South Africa was first described from Ceres and the nearby Prince Alfred hamlet (Blomefield & Geertsema 1990). After the species was known to have entered the country, reports of its presence in other regions quickly followed. It was therefore assumed that the moth had been spread from the Ceres region throughout the country by agricultural practices (Blomefield & Geertsema 1990, Blomefield *et al*. 1990). However, the low levels of gene diversity found within the Ceres population, especially in comparison with those of the populations from other stone fruit growing regions, indicate that the Ceres region is unlikely to be the centre of origin of the South African infestation. It is possible that *G. molesta* could have been present in other regions before being described from Ceres. The larvae of *G. molesta* are morphologically almost indistinguishable from those of the false codling moth *Thaumatotibia leucotreta*, which is extremely polyphagous and also known to attack stone fruit in South Africa (Newton 1998). It is therefore possible that initial *G. molesta* infestations in other parts of the country were wrongly identified as being *T. leucotreta*, allowing *G. molesta* infestations to go unnoticed in other stone fruit cultivating areas until the presence of the species was recognized in Ceres. Therefore, based on this as well as patterns of gene diversity and population clustering, it is suggested that the Ceres population originated from a different ancestral population, introduced at a different date or from a different location,

to that of populations from the remaining stone fruit cultivating areas in South Africa, which were derived from a common ancestral population.

Grapholita molesta populations from Caledon, Elgin, McGregor, Tulbagh and Stellenbosch were very closely related to each other. Estimates of population differentiation among these populations were calculated as G_{st} = 0.1519 with a corresponding *Nm* = 1.395, showing little evidence of population substructure. In addition it was apparent that populations from geographically close sites were not more similar than those separated by large distances and no correlation could be found between genetic and geographic distance. Indices of genetic identity varied between 0.9598 and 0.9841 between Caledon, Elgin, McGregor, Tulbagh and Stellenbosch populations. These values are only slightly lower than those calculated for populations of the closely related codling moth *C. pomonella* in France using allozyme analysis, which ranged between 0.995 and 1 (Buès & Toubon 1992). Reasons similar to those given for the uniformity of French *C. pomonella* populations can be invoked for the *G. molesta* population in South Africa, namely the heterogeneity of environmental conditions and the recent spread of stone fruit culture in South Africa. Since *G. molesta* was only recently introduced into South Africa, the high amount of gene flow that was calculated may also be reflective of recent shared ancestry. In addition, the spread of *G. molesta* populations by the movement of fruit, bins and nursery material between regions may also have contributed to range expansion by the species within a limited amount of time, leading to the formation of populations that are closely related genetically but are geographically distant.

Grapholita molesta populations over local scales were compared by collecting populations from orchards situated within one kilometre of each other. Analysis of molecular variance (AMOVA) indicated that genetic variation within populations was high, with most of the variation attributable to differences within populations. Variation among populations collected from adjacent orchards was significant, with 23% of the variation attributable to differences among populations. Results using principal co-ordinate analysis showed that these populations could be distinguished from each other using AFLP analysis. Therefore, it was concluded that *G. molesta* formed localized populations within South African stone fruit orchards, most likely as result of a low level of dispersal. These results are consistent with those obtained in Canada and the USA using mark-recapture techniques and may reflect a successful ecological strategy of the moth. Since a stone fruit orchard could be considered a stable habitat, it may be advantageous for *G. molesta* individuals to remain and oviposit within the orchard where it emerged. However, discordant individuals, apparently flying over larger distances between orchards, were also occasionally found. It is likely that these migrants and their progeny will be favoured in instances where fruit production fluctuates between seasons and a new habitat has to be sought. These individuals may also be responsible for the rapid dispersal that is often noted within an empty habitat (Blomefield *et al*. 1990).

In summary, it was found that *G. molesta* was introduced into South Africa at least twice and moth movement over large geographic distances was most likely mediated by human intervention, which continues to affect population structure. *G. molesta* dispersal between orchards in South Africa was limited, leading to the formation of localized populations. These results provide important information on the population genetics of *G. molesta* for designing and implementing sustainable pest management strategies.

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Tables

Table 6.1 Geographic location of *G. molesta* specimens from South Africa used for AFLP analysis.

Geographic location	Farm name	Latitude	Longitude	Orchard codes*
Elgin	Elgin Experimental Farm	34°9'S	19°2'E	-
	Fine Farms	34°13'S	19°4'E	6, 18
Caledon	Meulrivier	34°4'S	19°28'E	302, 307
McGregor	Rheebokskraal	33°58'S	19°50'E	-
Tulbagh	Roodezand	33°12'S	19°7'E	1E, 2B1, 4B, 6A, 11B, 19
Stellenbosch	Timberlea	33°55'S	18°52'E	F-block, Back Hill, V7, 20, Klipblok
	Tregurthen	33°55'S	18°56'E	Vlak, Huisblok1, Huisblok2
Ceres	Uitkoms	33°16'S	19°20'E	-

* if samples were analyzed from multiple orchards

Table 6.2. Genetic diversity indices among *G. molesta* populations sampled from six regions in South Africa, based on AFLP analysis.

	p (%)	*H*	N_R	N_{Pr}
Elgin	97.08	0.109	28	2
Caledon	99.43	0.186	71	15
McGregor	93.38	0.123	25	1
Tulbagh	98.70	0.170	45	5
Stellenbosch	98.87	0.156	53	7
Ceres	58.18	0.100	12	4
Population means	*90.94*	*0.141*	*39*	*5.66*
Total population	*100*	*0.182*	*111*	-

p (%) percent polymorphic loci, *H* Nei's gene diversity, N_R number of rare fragments and N_{Pr} number of private fragments

Table 6.3. Genetic distance and genetic identity indices (Nei 1978) for six South African *G. molesta* populations and a Canadian population, estimated using AFLP analysis.

	Elgin	Caledon	McGregor	Tulbagh	Stellenbosch	Ceres	Canada
Elgin	-	0.9753	0.9676	0.9598	0.9683	0.8250	0.7757
Caledon	0.0250	-	0.9647	0.9668	0.9721	0.8422	0.7956
McGregor	0.0329	0.0360	-	0.9670	0.9841	0.8610	0.8018
Tulbagh	0.0410	0.0338	0.0336	-	0.9815	0.8962	0.8284
Stellenbosch	0.0322	0.0283	0.0160	0.0187	-	0.8872	0.8164
Ceres	0.1923	0.1718	0.1496	0.1095	0.1197	-	0.8440
Canada	0.2540	0.2287	0.2209	0.1882	0.2029	0.1696	-

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 6.4. Analysis of molecular variance (AMOVA) for *G. molesta* populations sampled from orchards situated within one kilometre of each other, based on AFLP analysis.

	df	SS	MSS	Est. Var.	Total Variance (%)	*P*
Among populations						
Tulbagh (Roodezand)	4	168.27	42.07	5.09	20	< 0.001
Stellenbosch (Timberlea)	4	190.08	47.52	5.43	20	< 0.001
Stellenbosch (Tregurthen)	2	67.33	33.67	3.13	15	< 0.001
Total	12	583.63	48.64	6.13	23	< 0.001
Within populations						
Tulbagh (Roodezand)	16	333.92	20.87	20.87	80	< 0.001
Stellenbosch (Timberlea)	19	408.25	21.49	21.49	80	< 0.001
Stellenbosch (Tregurthen)	12	216.00	18.00	18.00	85	< 0.001
Total	47	958.17	20.39	20.39	77	< 0.001

Figures

Fig. 6.1. Locality map of the Western Cape province South Africa, showing *G. molesta* sample sampling sites.

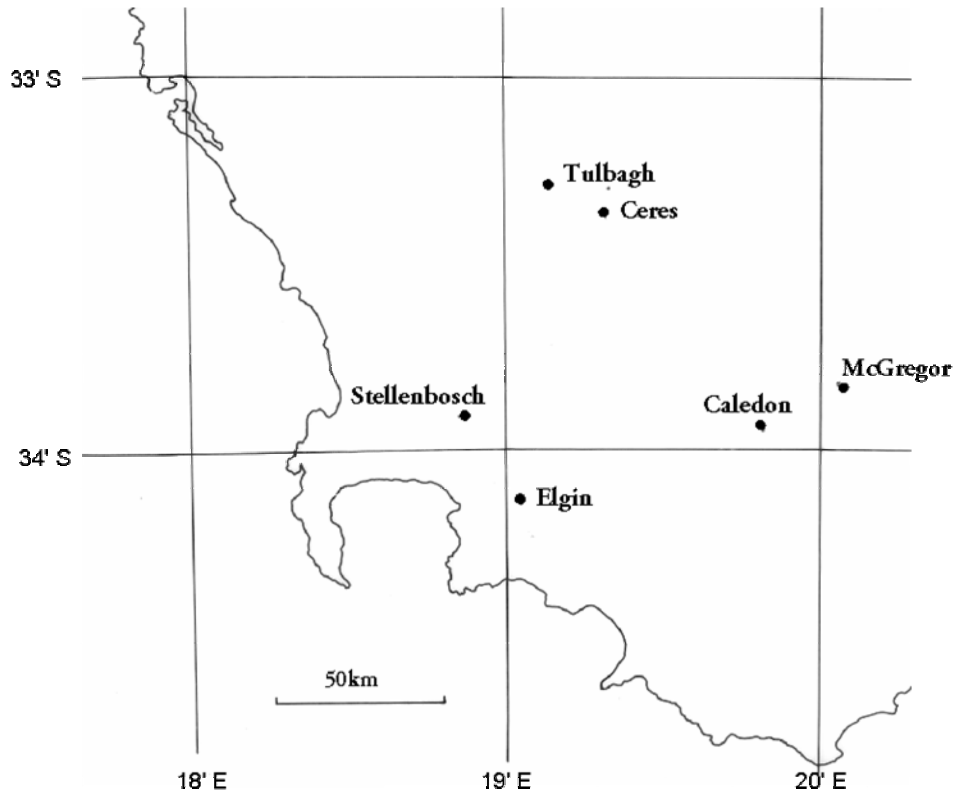


Fig. 6.2. Dendrogram (UPGMA) showing the relationships among six South African *G. molesta* populations from different geographic regions. This figure clearly shows the Ceres population as an outgroup to the remaining South African populations and the Canadian population as an outgroup to the South African population.

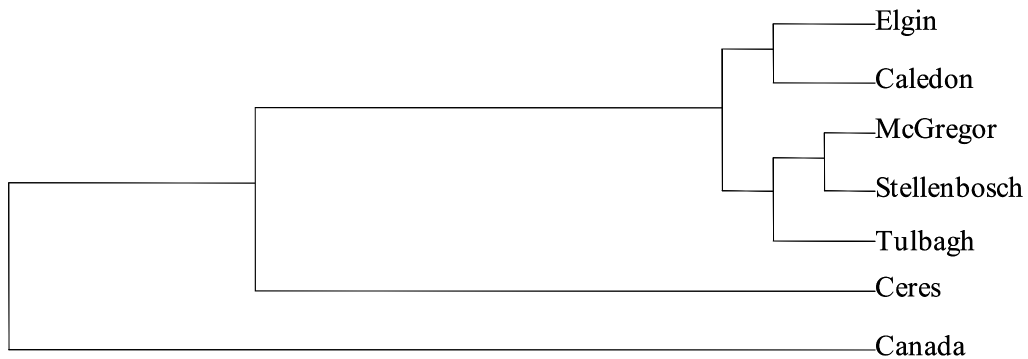
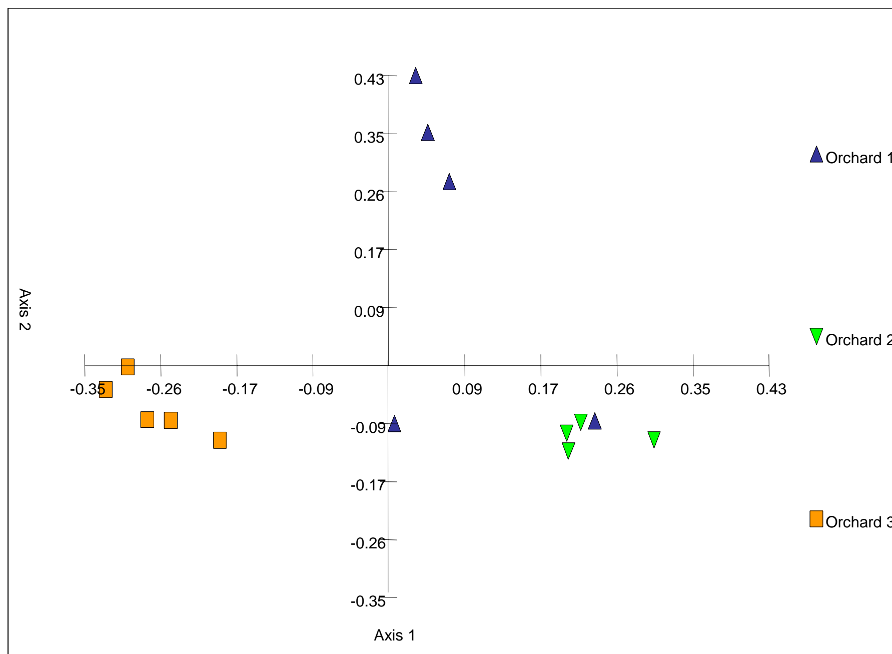


Fig. 6.3. Principal co-ordinate analysis showing the relationships among *G. molesta* populations sampled from three orchards within one kilometre of each other from Roodezand, Tulbagh, South Africa. Axes 1 and 2 describe 25.50% and 16.13% of the total variation respectively.



CHAPTER 7

Genetic variation among *Cydia pomonella* (Lepidoptera: Tortricidae) geographic and host populations in South Africa

7.1 Abstract

Information on genetic variation among geographic and host populations of *C. pomonella* L. (Lepidoptera: Tortricidae) in South Africa is lacking, despite the importance of these measures for the success of control practices such as chemical control and sterile insect release, which are affected by the amount of gene flow between populations. Therefore, populations sampled from nine geographically distant regions in South Africa from apples, pears and stone fruit were compared using amplified fragment length polymorphism (AFLP) with five selective primer pairs. Results showed that whereas populations from different hosts were not genetically differentiated, significant evidence for population substructure was apparent among geographic populations (G_{st} = 0.3778). Over local scales it was possible to ascribe individuals to populations based on their AFLP profiles. These results suggest that, while extensive gene flow occurs among populations from different hosts, gene flow among local geographic *C. pomonella* populations may be limited and are explained in terms of the limited flight range ability of moths, the relative isolation of pome fruit production areas and the absence of wild hosts.

7.2 Introduction

The codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) has been the key pest in South African pome fruit orchards since the species was first reported in the country in 1885 (Lounsbury 1898, Giliomee & Riedl 1998). The infestation potential of *C. pomonella* in South Africa is one of the highest in the world (Myburgh 1980) and moths may be active over as much as eight months of the year. *Cydia pomonella* causes extensive damage to apples and pears, with stone fruit being only occasionally attacked (Annecke & Moran 1982, Blomefield 1989). The use of insecticides remains the primary means of controlling *C. pomonella* in South African pome fruit orchards, with up to 11 different insecticides being used for control (Riedl *et al.* 1998). Extensive bioassays have shown that *C. pomonella* populations in South Africa have developed relatively high levels of resistance to the organophosphate azinphosmethyl and the pyrethroid insecticides (Blomefield 1994, Giliomee & Riedl 1998). Recently, the use of pheromone mating disruption has been implemented and the use of the sterile insect technique (SIT) is currently in its developmental phase for managing *C. pomonella* population levels in South Africa (Giliomee & Riedl 1998, Riedl *et al.*

1998, Addison 2005). The implementation and maintenance of these control practices at efficient levels requires an understanding of gene flow at various spatial levels, especially since they act over a longer period of time (Dorn *et al.* 1999).

Gene flow among *C. pomonella* populations has been investigated at least twice. Pashley & Bush (1979) compared *C. pomonella* populations, sampled from six different countries, using allozyme analysis and found little evidence for population substructure. Gene flow among *C. pomonella* populations on a more local scale was investigated in France and Switzerland, also using allozyme analysis (Buès & Toubon 1992, Buès *et al.* 1995). Geographic populations from these regions showed a high degree of genetic similarity, which was explained by the heterogeneity of environmental conditions undergone by populations and the relatively recent spread of apple culture in these regions. In an extension of this study, populations sampled from France from apple, pear, quince, walnut and apricot were compared (Buès *et al.* 1995). Few discernible differences were found among these populations and the hypothesis of local populations, some of which migrate to occasional hosts, was put forward. It is not known whether similar patterns of gene flow exist within South African *C. pomonella* populations.

The primary objectives of the present study were therefore to determine the extent of gene flow among and within *C. pomonella* geographic populations as well as among populations sampled from different hosts in South Africa. Amplified fragment length polymorphism analysis was chosen for analysis of *C. pomonella* populations since this marker has produced high resolution results for various lepidopteran species (for example Reineke *et al.* 1999, Salvato *et al.* 2002), including the closely-related oriental fruit moth *Grapholita molesta* (Chapter 6).

7.3 Material and methods

7.3.1 Insect material

Cydia pomonella specimens were sampled from nine different regions in South Africa (Table 7.1, Fig. 7.1). Populations were sampled from apples from the Warm Bokkeveld (November 2004) and Koue Bokkeveld (April 2004), from pears from Somerset West (March 2004) and Malmesbury (March 2004), from apples and pears from Elgin (January – February 2004), Stellenbosch (January 2004) and Vermaaklikheid (December 2004), from stone fruit from Worcester (December 2004) and from apples, pears and stone fruit from Tulbagh (February 2004, October 2004). Multiple populations were sampled from Elgin, Stellenbosch, Tulbagh, Vermaaklikheid and Warm Bokkeveld, from different farms or

orchards within the same farm. Specimens were sampled either by collecting infested fruit and allowing the moths to emerge or by using pheromone traps. Moths collected by pheromone traps were excluded from analysis of variation among host populations. A total of 129 *C. pomonella* specimens were included for analysis and voucher material of each specimen analyzed was deposited in the museum of the Department of Entomology, University of Stellenbosch.

7.3.2 DNA Analysis

AFLP analysis was conducted using standard procedures (Vos *et al.* 1995) with the restriction enzymes *Eco*RI and *Mse*I. DNA was extracted from the head and thorax of adult individuals, using a cetylmethylammonium bromide (CTAB) protocol (Reineke *et al*. 1998) and digested with five units of each restriction enzyme. Double-stranded adaptors were ligated to the resulting fragments and amplified using primers containing no selective nucleotides (*Eco*RI 5'- GAC TGC GTA CCA ATT C-3', *Mse*I 5'- GAT GAG TCC TGA GTA A-3'). A subset of these products was amplified with the incorporation of 0.25 μCi [γ-^{33}P]-dATP using primers each containing three selective nucleotides at the 3' end of the *Eco*RI and *Mse*I primers (*Eco*RI-GAC *Mse*I-GCA, *Eco*RI-CTG *Mse*I-CAT, *Eco*RI-AGT *Mse*I-CTA, *Eco*RI-ATC *Mse*I-CAC, *Eco*RI-ATC, *Mse*I-CAA). Amplification products were electrophoresed on 6% denaturing polyacrylamide gels for 2-3h. Gels were dried and visualized after exposure to autoradiographic film.

7.3.3 Data analysis

AFLP fragments were scored qualitatively to create binary matrices that were used in subsequent analyses. Gene diversity (*H*) (Nei 1987) was estimated using POPGENE version 1.31 (Yeh & Yang 1997). As an additional measure of genetic diversity, the percentage of polymorphic fragments (*p*) estimated at the 95% level was calculated. Population differentiation estimates (G_{st}) were calculated using POPGENE and these estimates were used to calculate the average number of individuals that migrate between populations per generation using the formula $Nm = \frac{1}{4}[(1/G_{st}) - 1]$ (Wright 1951).

Relationships between individuals were determined by constructing dendrograms using the unweighted pair group means arithmetic (UPGMA) based on Gower's general similarity coefficient (Sneath & Sokal 1963) using the software MVSP Version 3.11c (Kovach 1999). To evaluate the robustness of clusters in the UPGMA dendrograms the software WinBoot (Yap & Nelson 1996) was used to perform bootstrap analysis. Relationships among populations were determined by estimating indices of genetic identity (*I*) as well as genetic distance (*D*) (Nei 1978) using POPGENE. Genetic distances were used to view the

relationships among populations based on the construction of dendrograms using UPGMA analysis. Populations were also compared based on principal co-ordinate analysis (PCOA) using the software GenAlEx version 6 (Peakall & Smouse 2005). This software was also used to test the association between genetic distance and geographic distance using a Mantel test (Mantel 1967), with the number of permutations set at 1000.

7.4 Results

7.4.1 Genetic diversity

A total of 214 AFLP fragments were generated for analysis. Based on these data, genetic diversity was found to be high within the South African *C. pomonella* population (H = 0.1797). Only three AFLP fragments were monomorphic at the 95% level, resulting in 98.60% of the scored loci being polymorphic. Genetic diversity within regions varied significantly (Table 7.2), with four populations consistently below the population means for the total South African population, namely the Koue Bokkeveld, Malmesbury, Somerset West and Worcester populations.

7.4.2 Genetic differentiation among geographic populations

For nine South African *C. pomonella* geographic populations, comprising 129 individuals, population differentiation estimates of G_{st} = 0.3778 with a corresponding Nm = 0.4117 were calculated. Indices of genetic identity between populations ranged between 0.9894 (D = 0.0107) for the Elgin and Tulbagh populations and 0.8551 (D = 0.1566) for the Stellenbosch and Malmesbury populations (Table 7.3). A UPGMA dendrogram (Fig. 7.2) showed that populations clustered into four broad groups, with no apparent correlation between genetic and geographic distance. The Worcester, Somerset West and Malmesbury populations appeared to be differentiated from the remaining populations, with the latter two populations being more closely related to each other. The remaining populations separated into two groups, with the Stellenbosch, Tulbagh and Elgin populations forming a cluster separate to those sampled from the Warm and Koue Bokkeveld and Vermaaklikheid. A Mantel test confirmed that populations situated close together geographically were not always closely related genetically (r = 0.0719, $P > 0.05$).

Multiple *C. pomonella* populations were sampled from Elgin, Stellenbosch, Warm Bokkeveld, Tulbagh and Vermaaklikheid to assess variation over local scales. All calculated estimates of population

differentiation within regions indicated that the numbers of migrants exchanged among populations were insufficient to counter the effects of genetic drift, with G_{st} estimates ranging from 0.3420 in Elgin populations to 0.7012 in Tulbagh populations. It was possible to differentiate populations sampled from different farms within the same region as well as from orchards (in some instances less than one kilometre apart) within the same farm. An example is illustrated in Fig. 7.3, which shows the relationships among individuals sampled from three different orchards from Oak Valley farm, Elgin.

7.4.2 Genetic differentiation among host populations

Genetic variation among *C. pomonella* populations sampled from apples, pears and stone fruit was analyzed. Measures of population differentiation were calculated as G_{st} = 0.4324 when populations sampled only from apple orchards were analyzed and G_{st} = 0.5034 when populations sampled from only pears were analyzed. When populations from apples, pears and stone fruit were included in analyses, population differentiation was calculated as G_{st} = 0.5195. Analyses within regions yielded similar results, with values of population differentiation only slightly higher when hosts other than the principal host were included in the analyses. In addition, it was not possible to distinguish among populations sampled from different hosts using cluster analysis (data not shown).

7.5 Discussion

Measures of population differentiation between nine South African *C. pomonella* populations, based on AFLP analysis, indicated that populations were genetically differentiated with the number of migrants exchanged among populations insufficient to counter the effects of genetic drift. These results contrast with those obtained for *C. pomonella* populations from France and Switzerland, in which allozyme analysis indicated a large degree of genetic similarity between geographic populations (Buès & Toubon 1992). At least part of the discrepancy between results can be attributed to inherent properties of the markers used for analyses, since AFLP analysis has been shown to typically detect higher levels of genetic variation between closely-related populations than allozyme analysis (McMichael & Prowell 1999, Reineke *et al.* 1999). However, differences in *C. pomonella* history and habitat in South Africa and France could also have accounted for the differences in population structure observed between the two countries. Apple culture has only recently spread in France, which might have affected *C. pomonella* population structure (Buès & Toubon 1992). In contrast, apple culture was already well established in South Africa in 1885, when *C. pomonella* was first recorded in the country (Lounsbury 1898). In the South African population, two notable factors may also have contributed to the relatively low levels of gene flow among

populations, namely the relative isolation of pome fruit production regions as well as the absence of wild *C. pomonella* hosts, which serve as staging posts for dispersal over larger geographic areas.

Cluster analysis indicated that *C. pomonella* populations from South Africa that were situated close together geographically were not necessarily more closely related genetically than those situated further apart. This pattern of genetic variation could be due to human intervention in the form of fruit and seedling transport as well as the movement of bins (Higbee *et al.* 2001), which may have played an important role in the mixing of populations from distant geographic regions. *Cydia pomonella* populations sampled from Worcester, Somerset West and Malmesbury displayed low levels of gene diversity and were distinct to those from remaining South African populations. Since these regions are not major centers of pome fruit production, these populations may have become even more isolated than those from remaining regions. This could possibly have resulted in an increase in the effect of genetic drift on population structure.

Using AFLP analysis it was possible to distinguish between *C. pomonella* populations sampled from the same region as well as populations sampled from orchards situated less than one kilometre apart. These results suggest that gene flow between *C. pomonella* populations on a local scale is limited and may be related to the inherent dispersal ability of the moth. Individuals within *C. pomonella* populations vary genetically in their capacity to disperse over long distances (Schumacher *et al.* 1997a, Keil *et al.* 2001). The flight range of the majority of *C. pomonella* individuals has been found to be limited, with the species appearing to be fairly sedentary for a winged species (Borden 1931, Worthley 1932, Geier 1963, Vojnits 1972, Mani & Wildbolz 1977, Keil 2001). However, *C. pomonella* individuals may undertake single long flights of up to 11 km (Steiner 1940, Mani & Wildbolz 1977, Schumacher *et al.* 1997b). It has been suggested that the variation in dispersal capacity within populations of *C. pomonella* is adaptive in the context of its life history and habitat characteristics (Schumacher *et al*. 1997b). In modern orchards, which could be considered stable habitats, only short distance flights would be necessary for *C. pomonella* to reach another host plant and the most successful ecological strategy for the moth would be to stay within the habitat (Schumacher *et al*. 1997a,b). However, individuals with the capacity for a longer flight range may allow the population to escape unfavourable conditions and colonize new habitats (Southwood 1962, Wellington 1964). These individuals will be favoured in conditions where the food resource of the larvae (i.e. fruit) fluctuates. Factors such as the effect of alternate bearing, late frosts and early season pests have been identified as possible contributors to fruit fluctuation in pome fruit orchards (Schumacher *et al.* 1997b, Töpfer *et al*. 1999). However, the latter two factors are not relevant in the context of South African pome orchards, which may have resulted in an increased selection for sedentary genotypes. This does not preclude the possibility that individuals capable of long distance flights are found within populations since newly-established South African pome fruit orchards seldom remain free of *C. pomonella* infestation.

The high level of genetic variation among populations that was found on a local scale suggests that selection pressures by insecticide use will select for resistance at an orchard level. These results correlate well with studies of insecticide resistance in South African *C. pomonella* populations, where resistance appears to be highly localized.

Cydia pomonella populations were sampled from apples, pears and stone fruit in South Africa to assess the effect of different hosts on patterns of genetic variation. Results similar to those by Buès *et al*. (1995) for French *C. pomonella* populations were found, with estimates of population differentiation only slightly higher when populations from hosts other than apples were included in analyses. Also, it was also not possible to distinguish among populations sampled from different hosts. Buès *et al*. (1995) described these results in terms of local populations that emigrate to occasional hosts, with regular mixing of populations due to spatio-temporal synchronisms. It is likely that a similar effect is apparent in South African *C. pomonella* populations.

This study provides the first account of genetic variation among *C. pomonella* populations in South African orchards. The following can be concluded from the data (1) gene flow among geographic populations is limited, most likely as result of limited moth dispersal, the relative isolation of pome fruit production areas and the absence of uncultivated hosts and (2) populations sampled from different hosts do not appear to be genetically differentiated, suggesting regular gene flow among populations from different hosts as a result of spatio-temporal synchronisms. Insect dispersal and gene flow among populations may affect practices used for managing population levels of *C. pomonella.* For example, limited gene flow among populations may retard the spread of insecticide resistance at a local scale but may also spread resistance rapidly at the global level and should therefore be considered in insecticide resistance management programs (Han & Caprio 2002). Likewise, the design of successful pheromone mating disruption and SIT tactics must take into consideration limited organism dispersal for accurate determination of factors such as pheromone placement and the sites for release of sterile moths. The results of this study therefore have important implications for the successful management of *C. pomonella* in South Africa.

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Tables

Table 7.1. Geographic location of *C. pomonella* specimens from South Africa used for AFLP analysis.

Region	Farm	Host	Latitude	Longitude	Orchard codes*
Elgin	Arosa	Apples	34°15’S	19°4’E	-
	Braeview	Apples	34°14’S	19°4’E	-
	Elgin Experimental Farm	Apples	34°9'S	19°2'E	-
	Fine Farms	Apples	34°13'S	19°4'E	8, 22, 21B, 21A, 30
	Oak Valley	Apples	34°10’S	19°4’E	W17, A23, G9
	Lorraine Organic Farm	Apples	34°11’S	19°2’E	-
	Molteno Trust	Pears	34°8’S	19°3’E	62, 67
Stellenbosch	Timberlea	Apples / pears	33°55'S	18°52'E	V5, Back Hill, V1, V3, 10, 15
	Tregurthen	Apples	33° 55'S	18° 56'E	-
Warm Bokkeveld	Marceaux	Apples	19°19’S	33°21’E	1, 2, 3
	Vreeland	Apples	19°19’S	33°20’E	-
Koue Bokkeveld	Wesland	Apples	19°23’S	33°1’E	-
Tulbagh	Freeman	Apples	33°13’S	19.7’°E	-
	Roodezand	Stone fruit	33°12'S	19°7'E	-
	Twee Jonge Gezellen	Pears	33°15’S	19°7’E	-
	Vrolikheid	Apples	33°13’S	19°9’E	-
Somerset West	Vergelegen	Pears	34°4’S	18°53’E	-
Worcester	Fairview	Stone fruit	33°30’S	19°15’E	-
Malmesbury	Wynkeldershoek	Pears	33o26’S	18°47’E	-
Vermaaklikheid	-	Apples / pears	34°18’S	21°2’E	-

* if samples were analyzed from multiple orchards

Table 7.2. Measures of genetic diversity for nine *C. pomonella* populations sampled from South Africa, based on analysis of 214 AFLP fragments.

	p (%)	*H*
Elgin	98.85	0.162
Koue Bokkeveld	66.66	0.095
Malmesbury	35.23	0.059
Somerset West	35.80	0.055
Stellenbosch	97.19	0.182
Tulbagh	97.87	0.118
Vermaaklikheid	76.04	0.112
Warm Bokkeveld	89.05	0.137
Worcester	41.86	0.062
Population means	*70.95*	*0.109*
Total population	*98.60*	*0.180*

p (%) percent polymorphic loci, *H* Nei’s gene diversity

Table 7.3. Nei's unbiased measures of genetic identity and genetic distance (1978), based on AFLP analysis, for *C. pomonella* populations sampled from nine geographically distant regions in South Africa.

	Stellenbosch	Warm Bokkeveld	Koue Bokkeveld	Somerset West	Malmesbury	Tulbagh	Elgin	Worcester	Vermaaklikheid
Stellenbosch	-	0.9701	0.9347	0.8821	0.8551	0.9834	0.9827	0.8843	0.9423
Warm Bokkeveld	0.0304	-	0.9791	0.9260	0.9096	0.9643	0.9718	0.9172	0.9633
Koue Bokkeveld	0.0676	0.0211	-	0.9251	0.9186	0.9297	0.9460	0.9085	0.9664
Somerset West	0.1254	0.0769	0.0778	-	0.9177	0.8900	0.8872	0.8860	0.9119
Malmesbury	0.1566	0.0948	0.0849	0.0859	-	0.8657	0.8635	0.8735	0.9044
Tulbagh	0.0167	0.0364	0.0728	0.1165	0.1442	-	0.9894	0.8794	0.9456
Elgin	0.0175	0.0286	0.0555	0.1197	0.1468	0.0107	-	0.8923	0.9582
Worcester	0.1229	0.0865	0.0960	0.1210	0.1352	0.1285	0.1140	-	0.9306
Vermaaklikheid	0.0595	0.0373	0.0341	0.0922	0.1004	0.0559	0.0427	0.0719	-

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Figures

Fig. 7.1. Map of the Western Cape region showing the locations of *C. pomonella* sampling sites.

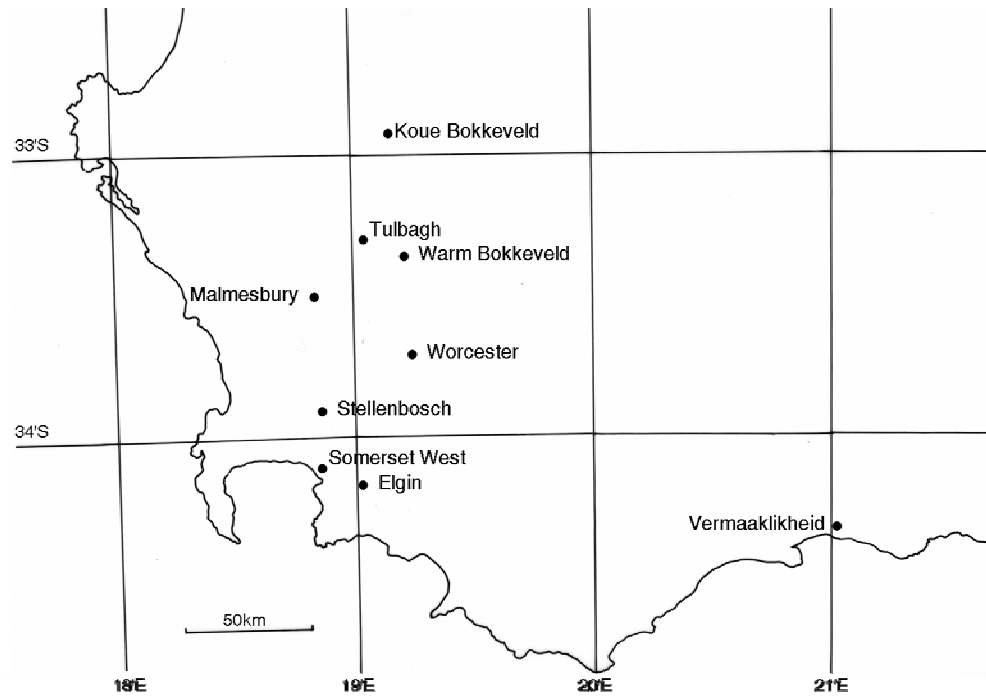

Fig. 7.2. Cluster analysis (UPGMA), based on AFLP analysis, showing the relationship among nine geographic *C. pomonella* populations sampled from South Africa.

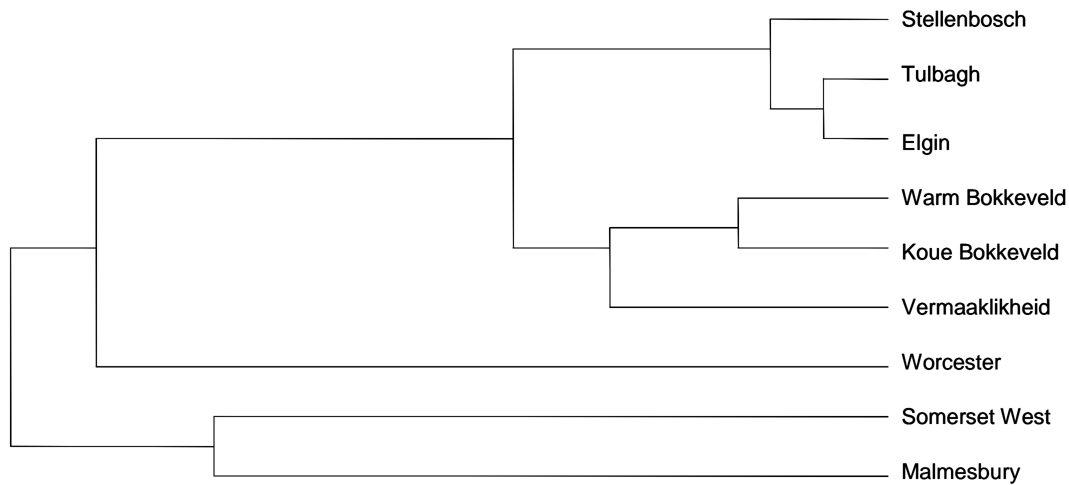

Fig. 7.3. Cluster analysis (UPGMA) showing the relationships between *C. pomonella* individuals sampled from three orchards (namely A23, G9 and W17) from Oak Valley, Elgin. The numbers next to the branches indicate the percent of support.

CHAPTER 8

Comparative analysis of population genetic structure of two closely related tortricid species feeding on macadamias and litchis in South Africa

8.1 Abstract

The macadamia nut borer *Thaumatotibia batrachopa* and the litchi moth *Cryptophlebia peltastica* are Afrotropical species causing extensive damage to cultivated macadamias and litchis in the northernmost provinces in South Africa and may also feed on uncultivated indigenous and exotic hosts. To analyse the population genetic structure of the two species, AFLP analysis was conducted. Patterns of genetic diversity in *C. peltastica* populations were contrasted in Mpumalanga province, where the species is controlled, with those in the Western Cape, where populations occur under more natural conditions. Results indicated that genetic diversity was high in *T. batrachopa* populations (H = 0.2219) with significant genetic differentiation among populations (G_{st} = 0.358). Genetic diversity was higher within *C. peltastica* Mpumalanga populations than Western Cape populations (H = 0.1906 and 0.1687 respectively). However, population differentiation was higher among Western Cape populations than Mpumalanga populations (G_{st} = 0.4124 and 0.3799 respectively). In both *T. batrachopa and C. peltastica* populations, geographic populations clustered together in the UPGMA analysis and extensive divergence was found over local scales. It is suggested that the population genetic structure of both species is influenced by limited dispersal among populations and that chemical control of *C. peltastica* in Mpumalanga province has not succeeded in reducing population size. This information is essential for the design of pest management strategies as well as in instances where these species may be introduced into other continents.

8.2 Introduction

Members of the family Tortricidae (Lepidoptera) form important components of the pest complex of macadamias and litchis in countries where these crops are grown commercially (Jones 1995). In South Africa three tortricids are of economic importance on macadamias and litchis, namely the macadamia nut borer *Thaumatotibia batrachopa* (Meyrick), the false codling moth *T. leucotreta* (Meyrick) and the litchi moth *Cryptophlebia peltastica* (Meyrick). All three species are Afrotropical and have not been recorded on any other continent although both *C. peltastica* and *T. leucotreta* are found on islands surrounding Africa (Meyrick 1930, Quilici 1988, Newton 1998). The biology of the three species is similar. The eggs are laid on the peel of the fruit and the newly emerged larvae bore into the pip or nut, where they develop.

Pupation usually takes place in the soil, but may also occur either in or on the fruit. Whereas *T. batrachopa* and *C. peltastica* cause economic injury only to litchis and macadamias, *T. leucotreta* has an extensive host range and is of economic importance on a diverse multitude of cultivated crops (Newton 1998). Therefore, only the two primary pests of litchis and macadamias, namely *T. batrachopa* and *C. peltastica*, will be considered in the current study.

Thaumatotibia batrachopa appears to be the dominant tortricid pest species on macadamias in South Africa, where it was first recorded as such in 1999. An unusual infestation occurred on citrus in 1972, but the population was soon eradicated and the species has not been recorded from citrus since (de Villiers 2001a). Four alternative *T. batrachopa* hosts have been identified in Malawi (La Croix & Thindwa 1986), but the species seems to be specific on macadamia in South Africa (de Villiers 2001a). *Cryptophlebia peltastica* is polyphagous and of economic importance on litchis and, to a limited extent, on macadamias. The species is found throughout South Africa on at least thirteen alternative hosts comprised mainly of indigenous ornamental tree species (Bradley 1953, Grové 1999, de Villiers 2001b). *Cryptophlebia peltastica* also forms part of the microlepidopteran assemblage in galls of *Ravenelia macowiana* (Uredinales) on *Acacia karroo* (McGeogh 1993, Kruger 1998) as well as in galls of *Uromycladium tepperianum* (Uredinales), used for the biological control of the invasive Port Jackson willow (*A. saligna*). Since extensive macadamia and litchi production is restricted to the northernmost provinces of South Africa, these are the only regions where *C. peltastica* population levels are actively controlled using insecticides (Nel *et al*. 1999). Elsewhere in South Africa, including in the Western Cape, *C. peltastica* forms part of the natural fauna.

Both *T. batrachopa* and *C. peltastica* have proved difficult to mass rear under laboratory conditions. As such, basic information on the ecology of the species, including factors such as gene flow and dispersal among populations, is not available. This information is not only essential for the successful implementation and maintenance of control practices, but also for instances where the species may be introduced accidentally onto other continents. The South African production of litchis and macadamias makes a considerable contribution to the worldwide production of these crops. Therefore, the possibility exists that either *T. batrachopa* or *C. peltastica* may be introduced accidentally into countries where they were not present before, despite strict quarantine measures. If this should occur, one of the most important factors that may affect the risk of establishment of *T. batrachopa* or *C. peltastica* in litchis, macadamias or alternative hosts is dispersal, as a population will only establish if a gravid female is able to disperse to a suitable host plant. Organism dispersal can be inferred from simple population genetic statistics such as gene flow measures since population genetic differentiation is influenced by the movement of individuals (Bohonak 1999). Molecular studies are able to provide long-term dispersal estimates over the geographic range of the species (Roderick 1996, Bohonak 1999), whereas traditional methods provide estimates of

dispersal over a known space and time. These studies compliment each other and are ideally used in conjunction (Roderick 1996). However, until protocols are described for mass rearing the two species, traditional studies of dispersal cannot be conducted for *T. batrachopa* or *C. peltastica*. Characterization of the population genetic structure of the two species is therefore the only tool available for gaining much-needed information on gene flow and dispersal among populations.

The aims of this study were therefore (1) to characterize the population genetic structure of *T. batrachopa* and *C. peltastica,* and (2) to contrast population genetic parameters between areas where *C. peltastica* populations occur naturally and where populations are actively controlled to gain further insight into the ecology of the species.

8.3 Material and Methods

8.3.1 Insect material

Specimens of *T. batrachopa* were sampled from macadamias from Mpumalanga (December 2003) and Limpopo provinces (June 2003) (Table 8.1, Fig. 8.1). Specimens of *C. peltastica* were collected from the Western Cape during September – December 2003 from *U. tepperianum* galls on *A. saligna*, which is the primary host in this region and from Mpumalanga province during December 2003 from litchis and macadamias. The majority of specimens were obtained by collecting infested fruit or galls and allowing the moths to emerge. However, *C. peltastica* specimens were also collected from pheromone traps placed in litchi orchards. Voucher material of specimens included for analysis was deposited in the museum of the Department of Entomology, University of Stellenbosch.

8.3.2 DNA Extraction

DNA was extracted from the heads and legs of moths using the CTAB-based protocol described by Reineke *et al.* (1998).

8.3.3 DNA Analysis

Amplified fragment length polymorphism (AFLP) analysis was performed based on standard radioactive procedures using the enzymes *Eco*RI and *Mse*I (Vos *et al.* 1995). Non-selective amplification was performed using primers containing no selective nucleotides (*Eco*RI 5'-GAC TGC GTA CCA ATT C-3', *Mse*I 5'-GAT GAG TCC TGA GTA A-3'). All primers chosen for selective amplification contained three

nucleotides at the 3' end (*Eco*RI-ATC *Mse*I-CTA, *Eco*RI-ATC *Mse*I-CAT, *Eco*RI-AGT *Mse*I-CTA, *Eco*RI-CTG *Mse*I-CAC, *Eco*RI-GAC, *Mse*I-GCA). *Eco*RI primers were labeled with the incorporation of 0.25 μCi [γ-^{33}P]-dATP and amplification products were electrophoresed on 6% denaturing polyacrylamide gels.

8.3.4 Data analysis

AFLP fragments were scored manually for presence or absence. Measures of genetic diversity included Nei's measure of gene diversity (*H*) (Nei 1973), calculated using the software POPGENE version 1.31 (Yeh & Yang 1997), as well as the number of rare fragments within populations (fragments that occurred in 10% or fewer of the individuals) and the number of private fragments (fragments that were unique to a population or region). As measures of population differentiation G_{st} estimates were calculated using POPGENE. Indices of genetic identity and genetic distance (Nei 1978) were calculated and the latter used for viewing the relationships between populations using cluster analysis based on the unweighted pair group means arithmetic (UPGMA) metric in POPGENE (Yeh & Yang 1997). Mantel tests (Mantel 1967) were conducted to test for the correlation between genetic and geographic distances using the software GenAlEx version 6 (Peakall & Smouse 2005). The relationships between individuals were determined using principal co-ordinate analysis based on Gower's similarity coefficient using the MultiVariate Statistical Package (Kovach 1999).

8.4 Results

8.4.1 Genetic diversity

A total of 219 AFLP fragments were scored for analysis of *T. batrachopa* populations and 262 for *C. peltastica* populations. Estimates of Nei's gene diversity (*H*) were calculated as 0.2219 in *T. batrachopa* populations and 0.1909 in *C. peltastica* populations (Table 8.2). A higher proportion of rare fragments was recorded in *C. peltastica* than *T. batrachopa* populations. Estimates of gene diversity in *C. peltastica* were lower in Western Cape populations (H = 0.1687) than in populations from Mpumalanga province (H = 0.1906). Western Cape *C. peltastica* populations possessed 24 AFLP fragments that were not found within the Mpumalanga populations and 18 AFLP fragments were present in Mpumalanga populations that did not occur in Western Cape populations. These private fragments were mainly found at a frequency of <20% although two fragments with frequencies >20% and a single fragment occurring in 36% of the individuals was present within the Western Cape population that was not present in Mpumalanga populations. Mpumalanga *C. peltastica* populations possessed a single fragment that occurred at a

frequency of >20% of the population but was absent in the Western Cape population. Similar proportions of rare fragments were recorded for *C. peltastica* populations from both provinces.

8.4.2 Genetic differentiation

8.4.2.1. Thaumatotibia batrachopa

Estimates of population differentiation were calculated as G_{st} = 0.3580 in *T. batrachopa* populations. Populations sampled from the Limpopo and Mpumalanga provinces were clearly separated, as shown by UPGMA cluster analysis (Fig. 8.2). Estimates of genetic identity ranged between 0.6621 (Levubu and Halls Boschrand, Nelspruit) and 0.9491 (Burgershall, Hazyview and Institute for Tropical and Subtropical Crops, Nelspruit). Estimates of genetic distance (Nei 1978) varied between 0.0522 (Burgershall, Hazyview and Institute for Tropical and Subtropical Crops, Nelspruit) and 0.4123 (Levubu and Halls Boschrand, Nelspruit) in the most divergent populations. Mantel tests indicated that genetic and geographic distance matrices were not significantly correlated in *T. batrachopa* populations ($r = 0.98$, $P > 0.05$).

8.4.2.2. Cryptophlebia peltastica

Principal co-ordinate analysis indicated that *C. peltastica* populations from the Western Cape and Mpumalanga provinces formed two separate clusters although some overlap was apparent between populations from these provinces (Fig. 8.3). A third cluster was formed by five individuals, all collected from a single orchard from Kaalrug farm, Malelane, Mpumalanga.

Measures of *C. peltastica* population differentiation within the Western Cape were calculated as G_{st} = 0.4124. Genetic distances between populations from the Western Cape varied between 0.0171 (Noordhoek and Simondium) and 0.1999 (Stellenbosch and Kylemore), with genetic similarity indices ranging between 0.8188 (Stellenbosch and Kylemore) and 0.9830 (Noordhoek and Simondium). Cluster analysis of six Western Cape populations (Fig. 8.4) showed that populations geographically situated close together were not always more closely related genetically than those situated further apart. A Mantel test conducted to establish the relationship between genetic and geographic distance found only a weak correlation between the two matrices ($r = 0.0704$, $P > 0.05$).

Estimates of population differentiation were lower in *C. peltastica* populations sampled from Mpumalanga province (G_{st} = 0.3799) than those sampled from the Western Cape. UPGMA cluster analysis indicated

that whereas populations sampled from Hazyview clustered together, those from Nelpruit were closely related to Malelane populations (Fig. 8.5). Genetic distances between populations sampled from Mpumalanga province ranged from 0.0318 (Mataffin East and Halls Boschrand, Nelspruit) to 0.1280 (Institute for Tropical and Subtropical Crops, Nelspruit and Burgershall, Hazyview). Indices of genetic similarity ranged from 0.8798 (Institute for Tropical and Subtropical Crops, Nelspruit and Burgershall, Hazyview) and 0.9687 (Mataffin East and Halls Boschrand, Nelspruit). Mantel tests indicated a weak correlation between genetic and geographic distance ($r = 0.0722$, $P > 0.05$).

8.5 Discussion

8.5.1 *Thaumatotibia batrachopa*

Thaumatotibia batrachopa only recently manifested itself as a pest in South African macadamia orchards and it is therefore likely that founder effects could have played a major role in shaping its population genetic structure. The reduced levels of genetic diversity in the Hazyview and Levubu populations may be the result of a series of founder effects reducing variation during range expansion of the species. Compared to these two regions, high genetic diversity was found within Nelspruit populations, in which more than twice the number of private fragments was recorded than in the Levubu and Hazyview populations combined. *Thaumatotibia batrachopa* populations showed significant subdivision, a high level of population differentiation ($G_{st} = 0.3580$) and extensive genetic divergence was found among populations sampled from farms separated by distances of as little as 2 km. These results indicate that gene flow among populations is low and may be due to limited dispersal between populations also evident in related tortricid species such as *Grapholita molesta* (Summers 1966, Sziraki 1979, Vickers *et al*. 1985, Chapter 6) and *Cydia pomella* (Borden 1931, Geier 1963, Vojnits 1972, Mani & Wildbolz 1977, Keil 2001, Chapter 7).

8.5.2 *Cryptophlebia peltastica*

Genetic diversity was higher within the *C. peltastica* Mpumalanga population ($H = 0.1687$) than within the Western Cape population ($H = 0.1906$). This result was unexpected since *C. peltastica* populations are exposed to insecticides in litchi orchards in Mpumalanga province. It is typical for insecticidal control to reduce the population size, causing genetic bottlenecks that decrease diversity and reduce the number of alleles (Nei *et al*. 1975). However, this does not seem to be the case in Mpumalanga populations, indicating that currently chemical control is not effective in reducing population size in *C. peltastica*

populations in litchi orchards. Alternative *C. peltastica* hosts are relatively abundant in Mpumalanga province. It is therefore possible that *C. peltastica* populations, maintained on these unsprayed hosts, are entering litchi orchards during the growing season, thereby increasing genetic diversity within the orchard.

Cryptophlebia peltastica populations sampled from the Western Cape and Mpumalanga provinces, compared using principal co-ordinate analysis, formed two separate clusters although considerable overlap was apparent among the respective populations. Those from the Western Cape populations possessed 24 AFLP fragments not found within the Mpumalanga populations and 18 AFLP fragments were present in Mpumalanga populations but not in the Western Cape population, although these private fragments were mainly found at a frequency of lower than 20%. The genetic differences found between populations sampled from the two provinces could be due to host differences, insecticide treatments, local climatic conditions or other unmeasured factors. Since large geographic distances separate the provinces from which populations were sampled, it may be necessary to examine populations in intervening provinces to investigate the possibility of a cline existing between the two provinces. Five individuals collected from a single orchard from Kaalrug farm, Malelane, formed a cluster distinct from those collected from the Western Cape and remaining Mpumalanga populations. Since the habitat in which these were collected was similar to those of the other populations sampled from the same farm, it is uncertain why all individuals from that particular orchard form a cluster separate to all other *C. peltastica* populations. Further analyses are necessary for elucidation.

Cryptophlebia peltastica populations sampled from the Western Cape showed a high degree of population differentiation (G_{st} = 0.4124). Measures of genetic distance showed extensive genetic divergence between populations over geographic distances of as little as one kilometre. The high level of population differentiation found may, in part, be due to the isolation of populations. *U. tepperianum* galls formed on *A. saligna* are the primary *C. peltastica* hosts in the Western Cape. However, *A. saligna* is the most important invasive plant threatening the conservation of biodiversity in the fynbos biome in South Africa (Macdonald & Jarman 1984) and this host is therefore actively removed as well as killed by *U. tepperianum* galls. The *C. peltastica* habitat in the Western Cape has therefore become fragmented, effectively isolating populations. *C. peltastica* population sizes may therefore be small, especially in areas where low levels of genetic diversity were found. This may have allowed genetic drift to influence genetic structure, increasing differentiation between populations and reducing genetic variation (Wright 1951, Lacy 1987, Milligan *et al*. 1994, Frankham 1996). Ultimately, the eradication of *A. saligna* in the Western Cape will result in the loss of a significant proportion of the genetic variation present in the South African *C. peltastica* population.

Cryptophlebia peltastica populations sampled from Mpumalanga province showed a slightly lower level of population differentiation than those sampled from the Western Cape (G_{st} = 0.3799). This may be due to factors such as the movement of infested fruit between areas, which may provide an explanation for why moths from geographically distant areas were more closely related genetically than those situated close together. However, similar to populations sampled from the Western Cape, it was possible to ascribe individuals to populations situated less than one kilometre apart based on their AFLP profiles.

The high levels of population differentiation among *C. peltastica* populations from agricultural and natural ecosystems as well as the extensive genetic divergence over local spatial scales suggest that, similar to *T. batrachopa, C. peltastica* dispersal is limited between populations. This information should be incorporated into existing pest management strategies for more efficient control of population levels. Also, in the event that either species is accidentally introduced into other continents, results indicate that dispersal from the initial point of introduction should be limited. Therefore, eradication procedures should be adapted accordingly.

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Tables

Table 8.1. Geographic location of *T. batrachopa* and *C. peltastica* specimens from South Africa used for AFLP analysis.

Species	Province	Region	Farm	Host	Latitude	Longitude
T. batrachopa	Limpopo	Levubu	-	Macadamia		
	Mpumalanga	Hazyview	Burgershall	Macadamia	31°4'S	25°5'E
		Nelspruit	Institute for Tropical & Subtropical Crops (ITSC)	Macadamia	30°58'S	25°26'E
			Friedenheim	Macadamia	30°59'S	25°27'E
			Halls Boschrand West	Macadamia	30°57'S	25°26'E
C. peltastica	Western Cape	Noordhoek	-	*A. saligna*	34°7'S	18°23'E
		Pniel	-	*A. saligna*	33°54'S	18°57'E
		Stellenbosch	-	*A. saligna*	33°57'S	18°50'E
		Simondium	-	*A. saligna*	33°51'S	18°58'E
		Paarl	-	*A. saligna*	33°44'S	18°60'E
		Kylemore	-	*A. saligna*	33°55'S	18°57'E
	Mpumalanga	Nelspruit	Mataffin	Litchi	30°56'S	25°27'E
			Halls Boschrand West	Litchi	30°57'S	25°26'E
			Institute for Tropical & Subtropical Crops (ITSC)	Macadamia	30°58'S	25°26'E
		Hazyview	Rooigom	Litchi	31°4'S	25°2'E
			Burgershall	Litchi	31°4'S	25°5'E
		Malelane	Kaalrug	Litchi	31°32'S	25°37'E

* if samples were collected from multiple orchards

Table 8.2. Genetic diversity estimates for populations of *T. batrachopa* and *C. peltastica* sampled from South Africa, based on AFLP analysis.

Species	Site	No bands	*p (%)*	*H*	N_R	N_{Pr}
T. batrachopa	Levubu	103	-	-	9	9
	Hazyview	149	73.83	0.1552	9	13
	Nelspruit	189	88.88	0.2104	29	50
	Total population	*219*	*91.3*	*0.2219*	*47*	-
C. peltastica	Noordhoek	147	83.67	0.1258	13	1
	Pniel	121	87.60	0.1112	11	1
	Stellenbosch	93	50.53	0.0740	12	3
	Simondium	180	85.56	0.1396	37	2
	Paarl	163	90.80	0.1771	32	4
	Kylemore	73	-	-	1	2
	Western Cape	*243*	*93.00*	*0.1687*	*99*	*24*
	Nelspruit	196	88.27	0.1748	40	4
	Hazyview	133	74.44	0.1217	16	2
	Malelane	200	95.00	0.1653	48	6
	Mpumalanga	*237*	*97.00*	*0.1906*	*98*	*18*
	Total population	*262*	*97.33*	*0.1909*	*95*	-

p (%) percent polymorphic loci, *H* gene diversity, N_R number of rare fragments, N_{PR} number of private fragments

Figures

Fig. 8.1. Map of South Africa showing *T. batrachopa* and *C. peltastica* sample collection sites.

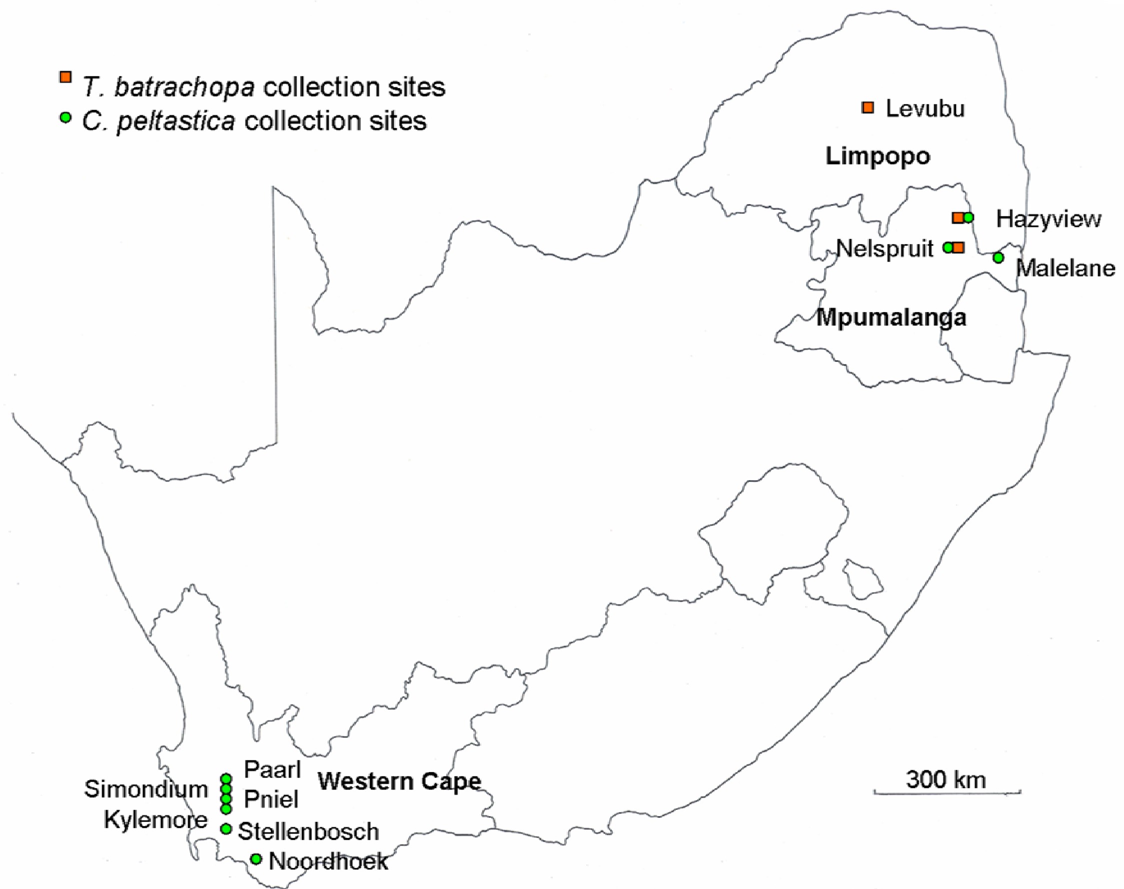
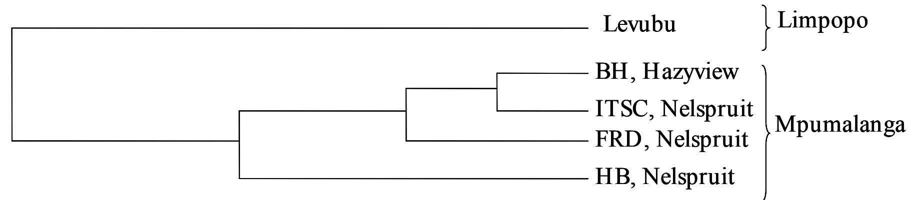


Fig. 8.2. Cluster analysis (UPGMA) of *T. batrachopa* populations, estimated using AFLP analysis, showing a clear distinction between populations sampled from the Limpopo and Mpumalanga provinces, South Africa.



BH Burgershall, ITSC Institute for Tropical and Subtropical Crops, FRD Friedenheim, HB Halls Boschrand

Fig. 8.3. Principal co-ordinate analysis showing the relationship between *C. peltastica* populations sampled from the Western Cape and Mpumalanga provinces, South Africa.

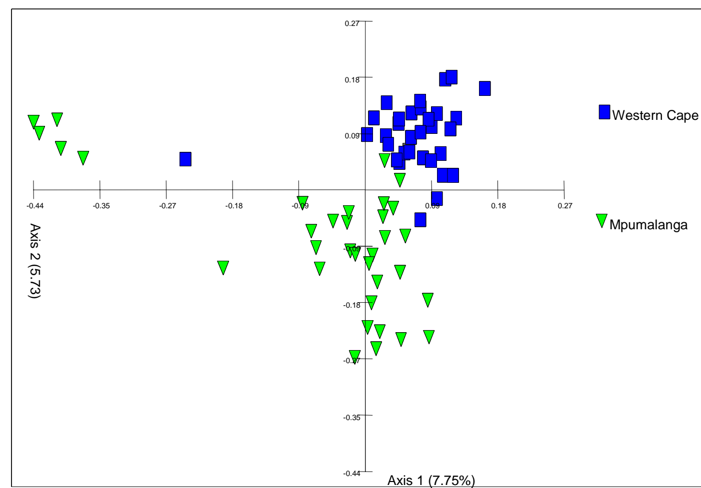


Fig. 8.4. UPGMA dendrogram showing the relationship among six *C. peltastica* populations sampled from the Western Cape province, South Africa.

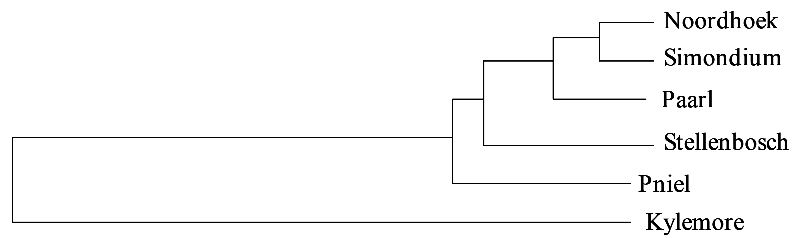
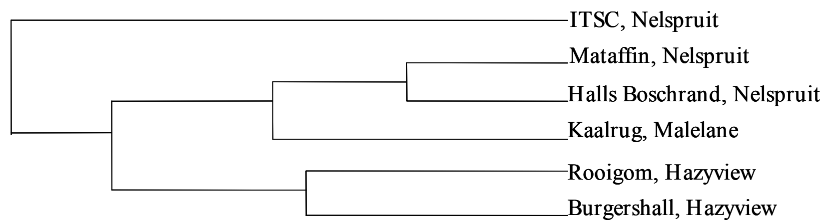


Fig. 8.5. UPGMA cluster analysis of *C. peltastica* populations sampled from Nelspruit, Hazyview and Malelane, Mpumalanga province, South Africa.



CHAPTER 9

Genetic variation among populations of false codling moth *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae), estimated by AFLP analysis, in South Africa

9.1 Abstract

The false codling moth *Thaumatotibia leucotreta* is one of the most destructive and polyphagous agricultural pests in Africa. Despite its economic importance, knowledge of genetic variation between geographic and host populations, which can be used to infer gene flow and insect dispersal, is lacking. This study examined the extent of genetic variation between populations sampled from citrus, apples, pears, plums, litchis, macadamias, star fruit and acorns from nine geographically distant regions in South Africa. Amplified fragment length polymorphism (AFLP) analysis using five selective primer pairs was used to generate 322 fragments for analysis of 163 individuals. Analysis of genetic variation among populations indicated restricted gene flow among geographic populations (G_{st} = 0.2929) but not among populations sampled from different hosts (G_{st} = 0.1193). Extensive divergence was found among populations sampled from agricultural systems from local geographic scales, suggesting decreased gene flow as a result of limited dispersal. These results may affect practices used to manage *T. leucotreta* population levels in agricultural systems and as such should be incorporated into existing pest management programs.

9.2 Introduction

Analysis of population genetic variation may offer insight into dispersal and gene flow. Since many practices used for managing insect pests in agricultural ecosystems may be affected by these factors, analysis of genetic variation is of primary importance for economically important pest species. Analysis of genetic variation has ultimately aided the management of various lepidopteran species of economic importance (for example Korman *et al*. 1993, Reineke *et al*. 1999, Han & Caprio 2002, Salvato *et al.* 2002) and may contribute towards improved control of the false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae).

Thaumatotibia leucotreta is indigenous to sub-Saharan Africa and one of the most economically important agricultural pests in Africa and surrounding islands (Stofberg 1954, Catling & Aschenborn 1974, Commonwealth Institute of Biological Control 1984, Newton 1998). The major factor contributing to the economic significance of *T. leucotreta* is the extensive host range of the larvae. In South Africa alone at least 21 cultivated and 14 indigenous wild host plants have been recorded (Schwartz 1981). Of the cultivated crops, *T. leucotreta* appears to prefer citrus although crops such as deciduous, tropical and subtropical fruit may also be heavily attacked (Blomefield 1989, Newton 1998). In tropical Africa *T. leucotreta* is a serious pest of cotton and maize (Angelini & Labonne 1970, Reed 1974). In South Africa *T. leucotreta* is active throughout the year in areas with a continual supply of fruit (Newton 1998). *T. leucotreta* eggs are usually laid on the fruit or foliage and larval development is completed inside the fruit, usually causing fruit abscission (Catling & Aschenborn 1974, Daiber 1978). Pupation usually takes place in the soil but may also take place inside the fruit (Gunn 1921, Stofberg 1954, Georgala 1969). As most of the life stages are inaccessible, chemical control against *T. leucotreta* is difficult. In addition, *T. leucotreta* has developed resistance to the benzyl-ureas that are commonly used for control (Hofmeyr & Pringle 1998). Varied results have been obtained using practices such as orchard sanitation, augmentative biological control and use of granulosis virus and these measures cannot be used as stand-alone tactics (Schwartz *et al*. 1982, Newton 1988, Newton 1989, Newton & Odendaal 1990, Jehle *et al*. 1992, Chambers *et al*. 1995, Moore *et al*. 2004). Therefore, the use of the sterile insect technique (SIT) has been evaluated for suppressing population levels of *T. leucotreta* (Schwartz 1979) and the commercial use of the technique is currently in its developmental phases (Bloem *et al*. 2003). However, for the implementation of SIT and more efficient use of alternative control practices, knowledge of patterns of gene flow and dispersal dynamics, which can be derived from the analysis of genetic variation among populations, is essential.

The primary aim of this study was therefore to use AFLP analysis to determine the extent of genetic variation among South African *T. leucotreta* populations from different regions as well as host species and to use this information to provide insight into population gene flow and dispersal dynamics.

9.3 Material and methods

9.3.1. Insect material

Specimens of *T. leucotreta* were obtained by means of pheromone traps or by collecting infested fruit and allowing the moths to emerge. Populations were sampled from three provinces in South Africa, namely

the Western Cape, Eastern Cape and Mpumalanga (Table 9.1, Fig. 9.1). From the Western Cape, populations were sampled from Citrusdal (May 2003), Stellenbosch (May – June 2003), Elgin (November – December 2003), Tulbagh (October 2004), Paarl (July 2003) and Retreat (May 2003). From the Eastern Cape, populations were sampled from the Sundays River Valley (June 2004). From Mpumalanga, populations were sampled from Nelspruit (December 2003), Hazyview (December 2003) and Malelane (December 2003). Individuals collected from a laboratory colony maintained in Britain were used as an outgroup to the South African populations. Voucher material of each of the 163 specimens included for analysis was deposited in the museum of the Department of Entomology, University of Stellenbosch.

9.3.2. AFLP analysis

DNA was extracted from the head and legs of moths or, in rare instances, the abdomens of larvae using the CTAB-based protocol described by Reineke *et al.* (1998), with reduced volumes. The AFLP procedure was performed using standard techniques (Vos *et al.* 1995). DNA was digested with the enzymes *Eco*RI and *Mse*I and pre-amplification of DNA templates was performed with primers containing no selective nucleotides (*Eco*RI 5'-GAC TGC GTA CCA ATT C-3', *Mse*I 5'-GAT GAG TCC TGA GTA A-3'). Selective amplifications were performed with ^{33}P-labelled *Eco*RI primers. Five combinations of selective primers were used, each containing three selective nucleotides (*Eco*RI-ATC *Mse*I-CTA, *Eco*RI-ATC *Mse*I-CAT, *Eco*RI-AGT *Mse*I-CTA, *Eco*RI-CTG *Mse*I-CAC, *Eco*RI-GAC, *Mse*I-GCA). Samples amplified with different primer combinations were loaded onto 6% denaturing polyacrylamide gels and electrophoresed for 2-3 hours. Gels were dried and exposed to autoradiographic film.

9.3.3. Data analysis

AFLP profiles were recorded in a binary matrix using "1" to denote fragment presence and "0" to denote fragment absence. The extent of genetic diversity within each population was estimated by calculating the number of polymorphic loci at the 95% level as well as the average gene diversity over all AFLP loci (*H*). The G_{st} coefficient, obtained using POPGENE version 1.31 (Yeh & Yang 1997), was used as a measure of genetic differentiation among populations. G_{st} coefficients were used to calculate gene flow, defined as the average number of individuals that migrate to each population per generation (*Nm*), using the equation $Nm = ¼ [(1/G_{st}) – 1]$ (Wright 1951). The relationships between populations were determined based on pair-wise measures of genetic distance (*D*) and genetic identity (*I*) calculated using Nei's (1978) unbiased genetic distance algorithm with the software POPGENE version 1.31 (Yeh & Yang 1997). Genetic distances

were used to produce a graphic representation of the relationships among populations by constructing a dendrogram based on the unweighted pair group method with arithmetic averages (UPGMA). The relationships between individuals were also viewed with Q-mode principal co-ordinate analysis (PCOA), based on a dissimilarity matrix generated using Gower's general similarity co-efficient (Sneath & Sokal 1963), with MVSP Version 3.11c (Kovach 1999). The association between genetic distance and geographic distance matrices was determined using a Mantel test (Mantel 1967), performed with the software GenAlEx version 6 (Peakall & Smouse 2005), with the number of permutations set at 1000.

9.4. Results

9.4.1. Genetic diversity

Analysis of 322 AFLP fragments indicated that genetic diversity within the South African *T. leucotreta* population was high (H = 0.1490). A total of 300 fragments (93.167%) were polymorphic whereas 138 fragments (40.30%) were rare, occurring at frequencies of 10% or fewer in the total population. Genetic diversity appeared to be higher within the Western Cape population (H= 0.1599) than both the Eastern Cape population (H = 0.1136) and the Mpumalanga population (H = 0.1390). Within regions, genetic diversity estimates ranged between H = 0.0842 in Malelane populations to H = 0.1549 in Citrusdal populations (Table 9.2).

9.4.2. Genetic differentiation among geographic populations

Genetic variation estimates for *T. leucotreta* populations sampled from three different provinces in South Africa, namely the Western Cape, Eastern Cape and Mpumalanga were calculated as G_{st} = 0.2929 (Nm = 0.604). Cluster analysis using the unweighted pair group means arithmetic (UPGMA) indicated that populations sampled from the same province were generally more closely related (Fig. 9.2) based on genetic distance estimates (Nei 1978) (Table 9.3). Populations sampled from Mpumalanga and the Eastern Cape appeared to be more closely related to each other than to those from the Western Cape. Population differentiation values were calculated as G_{st} = 0.2601 for Western Cape populations, G_{st} = 0.2036 for Eastern Cape populations and G_{st} = 0.2591 for Mpumalanga populations. The relationships between genetic and geographic distances, determined using Mantel tests, indicated non-significant correlations between the two matrices ranging from r =0.8607 (P = 0.833) in Mpumalanga populations to r = 0.0421 (P

= 0.923) in Western Cape populations. Extensive divergence was found between closely situated populations within regions and individuals could be more or less ascribed to populations based on their AFLP profiles. Within most regions from which populations were sampled from multiple sites, the estimated number of migrants exchanged among populations was too few to counter the effects of genetic drift ($Nm < 1$) although the estimated numbers of migrants approached one in populations sampled from the Sundays River Valley. The exception was the Retreat populations, where more than one migrant was exchanged between populations ($Nm = 1.15$).

9.4.3. Genetic differentiation among host populations

Genetic variation between populations sampled from different hosts was assessed by analyzing populations sampled from citrus, apples, pears, plums, litchis, macadamias, star fruit and acorns. Estimates of genetic differentiation based on host populations were calculated as $G_{st} = 0.1193$ ($Nm = 1.8452$). At regional and local scales, estimates of population differentiation were not significantly higher when populations from more hosts were included in analyses and it was not possible to distinguish among populations sampled from different hosts using cluster analysis (Fig. 9.3).

9.5. Discussion

9.4.1. Genetic diversity

Genetic diversity in *T. leucotreta* populations was high in every region and province in South Africa from which samples were collected. This included regions in which *T. leucotreta* was considered to be accidentally introduced. For example, populations from Stellenbosch and Citrusdal, introduced in 1947 and 1974 respectively (Giliomee & Riedl 1998, Newton 1998), had among the highest estimates of genetic diversity recorded in populations. These populations therefore do not appear to have suffered as result of population bottlenecks resulting from founder effects, which usually decrease genetic diversity. This high level of genetic diversity may partly explain the success of *T. leucotreta* in a variety of habitats.

9.5.2. Genetic differentiation among geographic populations

Based on AFLP analysis it was established that geographic populations of *T. leucotreta* in South Africa were genetically differentiated. Populations sampled from different sites within the same region generally tended to be more closely related to each other than to populations sampled from different regions. The "misplaced" populations on the dendrogram may most likely be due to the complex patterns of introduction into previously uncolonized regions and the movement of infested fruit between regions. The formation of these genetically distinct geographic populations may partly be responsible for the differences in infestation observed among regions (Newton 1985).

Over local scales, highly distinct populations were found and in certain instances where populations were separated by less than one kilometre, individuals could be ascribed to one or other population on the basis of their AFLP profiles. These results are similar to those produced for *Grapholita molesta* (Busck) (Chapter 6), *Cydia pomonella* (L.) (Chapter 7), *T. batrachopa* (Meyrick) (Chapter 8) and *Cryptophlebia peltastica* (Meyrick) (Chapter 8), even though *T. leucotreta* has a far more extensive host range than any of these species. The host range of a species may affect its genetic structure by providing staging posts for dispersal between populations or restricting populations to areas where the hosts occur. However, host range does not appear to have had a major effect on the population genetic structure of *T. leucotreta* in South African orchards. It is likely that, similar to the aforementioned species and the majority of animal species in nature (Bohonak 1999, Lowe *et al.* 2004), the population genetic structure of *T. leucotreta* may be affected by dispersal.

Thaumatotibia leucotreta has been described as a poorly dispersing species on the basis of mark-recapture studies and general field observations (Newton 1998). However, *T. leucotreta* males have been found to respond to females more than a kilometre away (Omer-Cooper 1939) and females were found to disperse up to 35m away to lay their eggs on sentinel fruits placed in an effectively empty habitat of non-bearing trees (Schwartz 1981). In this study, the only region in which significantly high levels of gene flow were calculated between populations was Retreat, the only urban area from which *T. leucotreta* samples were collected. It was not possible to distinguish between Retreat populations situated up to 6 km apart. It is therefore suggested that, like the closely related *C. pomonella* and *G. molesta*, *T. leucotreta* individuals may vary genetically in their capacity to disperse over long distances, which may be related to the habitat in which they are found (Mani & Wildbolz 1977, Sziraki 1979, Vickers *et al*. 1985, Rothschild & Vickers 1991, Schumacher 1997, Keil 2001). In orchards, where only short distance flights are required for *T. leucotreta* to reach another host plant, and where host plants are long-lived, the most successful ecological

strategy for the moth would be stay within the habitat. This would allow individuals to avoid the considerable risks associated with long-range dispersal, which includes the likelihood of not locating alternate resources and the increased probability of predation (Hardie *et al.* 2001, Weisser 2001). The same may not be relevant for *T. leucotreta* populations in urban environments, where the habitat is more variable than in orchards. A more thorough investigation of the genetic structure of *T. leucotreta* in urban or more natural populations may therefore be of considerable value.

9.5.3. Genetic differentiation among host populations

No evidence was found to suggest that populations sampled from citrus, apples, pears, plums, litchis, macadamias, star fruit and acorns were genetically differentiated despite early suggestions that *T. leucotreta* races having different host preferences may exist (Ford 1934, Omer-Cooper 1939). The implication of these results is that uncultivated hosts may maintain populations at times when fruit is unavailable in the orchard, confirming suggestions that the proximity of other susceptible cultivated or wild fruits has a considerable influence on the degree of *T. leucotreta* infestation (Gunn 1921, Daiber 1981, Anderson 1986). In addition, populations maintained on uncultivated hosts may affect the efficiency of chemical control and the development of insecticide resistance by maintaining reservoirs of susceptible populations and should be considered during the design of pest management programs.

9.6 Conclusion

This study provides the first account of genetic variation in *T. leucotreta* populations. From the data the following conclusions can be drawn: (1) *T. leucotreta* populations from different regions in South Africa are genetically differentiated, (2) restricted levels of gene flow occurs among populations from closely situated orchards, confirming limited dispersal in agricultural systems and (3) populations sampled from different hosts are not genetically differentiated, indicating that individuals may move freely between hosts. These results may affect practices used to manage *T. leucotreta* population levels and as such should be incorporated into existing pest management systems. In addition, since dispersal is one of the most important factors that may affect the risk of establishment of introduced species, these results may be useful in cases where the species is accidentally introduced into other countries, which could be potentially devastating considering the extensive host range of the larvae.

9.7 References

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Tables

Table 9.1. Geographic location of *T. leucotreta* specimens from South Africa used for AFLP analysis.

Province	Region	Farm	Host (s)	Latitude	Longitude
Western Cape	Citrusdal	Biesievlak	Citrus	33°56’S	18°51’E
		Boontjiesrivier	Citrus	32°34’S	19°1’E
		Hexrivier Blikhuis	Citrus	32°23’S	18°57’E
		Petersfield	Citrus	32°33’S	18°59’E
		Rivierplaas	Citrus	32°37’S	19°1’E
		Theerivier	Citrus, acorns	32°49’S	19°4’E
		Wilhelm Soete Trust	Citrus	32°43’S	19°3’E
	Stellenbosch	(University campus)	Acorns	33°56’S	18°52’E
		(Onderpapegaaiberg)	Unknown	33°56’S	18°52’E
		Timberlea	Pears	33°55'S	18°52'E
	Retreat	-	Unknown	34°3’S	18°29’E
	Elgin	Bellevue Experimental Farm	Apples	34°8’S	19°1’E
	Tulbagh	Twee Jonge Gezellen	Pears	33°15’S	19°7’E
		Roodezand	Plums	33°15’S	19°7’E
	Paarl	-	Unknown	33°45’S	18°59’E
Eastern Cape	Sundays River Valley	Woodridge	Citrus	33°28’S	25°41’E
		Penhill	Citrus	33°34’S	25°41’E
		Mfuleni	Citrus	33°27’S	25°32’E
		Carden	Citrus	33 °28’S	25°40’E
Mpumalanga	Nelspruit	Institute for Tropical and Subtropical Crops (ITSC)	Citrus, litchis, macadamias, star fruit	30°58’S	25°26’E
		Friedenheim	Litchi	30°59’S	25°27’E
		Mataffin East	Litchi	30°56’S	25°27’E
		Halls Boschrand West	Litchi	30°57’S	25°26’E
	Hazyview	Rooigom	Litchis	31°4’S	25°2’E
	Malelane	Institute for Tropical and Subtropical Crops (ITSC)	Litchis	31°33’S	25°26’E
		Kaalrug	Litchi	31°32’S	25°37’E

* if samples were sampled from multiple orchards

Table 9.2. Genetic diversity estimates calculated for *T. leucotreta* populations sampled from South Africa, based on analysis of 322 AFLP fragments.

Province	Region	No. bands	*p* (%)	*H*
Western Cape	Citrusdal	290	90.00	0.1549
	Stellenbosch	275	92.36	0.1502
	Retreat	201	81.09	0.1253
	Elgin	177	80.23	0.1172
	Paarl	166	76.51	0.1163
	Tulbagh	154	82.47	0.1099
	Total	318	92.77	0.1599
Eastern Cape	Sundays River Valley	212	89.15	0.1136
Mpumalanga	Nelspruit	245	86.53	0.1514
	Hazyview	198	80.81	0.1116
	Malelane	182	79.12	0.0842
	Total	279	87.10	0.1390
Total population		322	93.167	0.1490

p (%) percent polymorphic loci, *H* Nei's gene diversity

Table 9.3. Genetic distance and genetic identity indices (Nei 1978), estimated using AFLP analysis, for nine South African *T. leucotreta* populations and a British population.

	Citrusdal	Stellenbosch	Retreat	Paarl	Elgin	Nelspruit	Hazyview	Malelane	Sundays River Valley	British lab colony
Citrusdal	-	0.9905	0.9878	0.9809	0.9768	0.9843	0.9769	0.9746	0.9785	0.9160
Stellenbosch	0.0095	-	0.9812	0.9758	0.9741	0.9840	0.9720	0.9731	0.9774	0.9181
Retreat	0.0123	0.0189	-	0.9789	0.9751	0.9739	0.9748	0.9720	0.9695	0.9250
Paarl	0.0193	0.0245	0.0214	-	0.9723	0.9732	0.9747	0.9697	0.9673	0.9202
Elgin	0.0235	0.0263	0.0252	0.0281	-	0.9802	0.9780	0.9754	0.9670	0.9187
Nelspruit	0.0159	0.0161	0.0264	0.0272	0.0200	-	0.9828	0.9808	0.9798	0.9230
Hazyview	0.0233	0.0284	0.0256	0.0257	0.0222	0.0173	-	0.9925	0.9786	0.9210
Malelane	0.0258	0.0273	0.0284	0.0308	0.0249	0.0193	0.0075	-	0.9820	0.9187
Sundays River Valley	0.0218	0.0228	0.0309	0.0333	0.0336	0.0204	0.0216	0.0182	-	0.9183
British lab colony	0.0877	0.0854	0.0780	0.0832	0.0848	0.0802	0.0823	0.0848	0.0852	-

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Figures

Fig. 9.1. Locality map of South Africa, showing *T. leucotreta* sample collection sites.

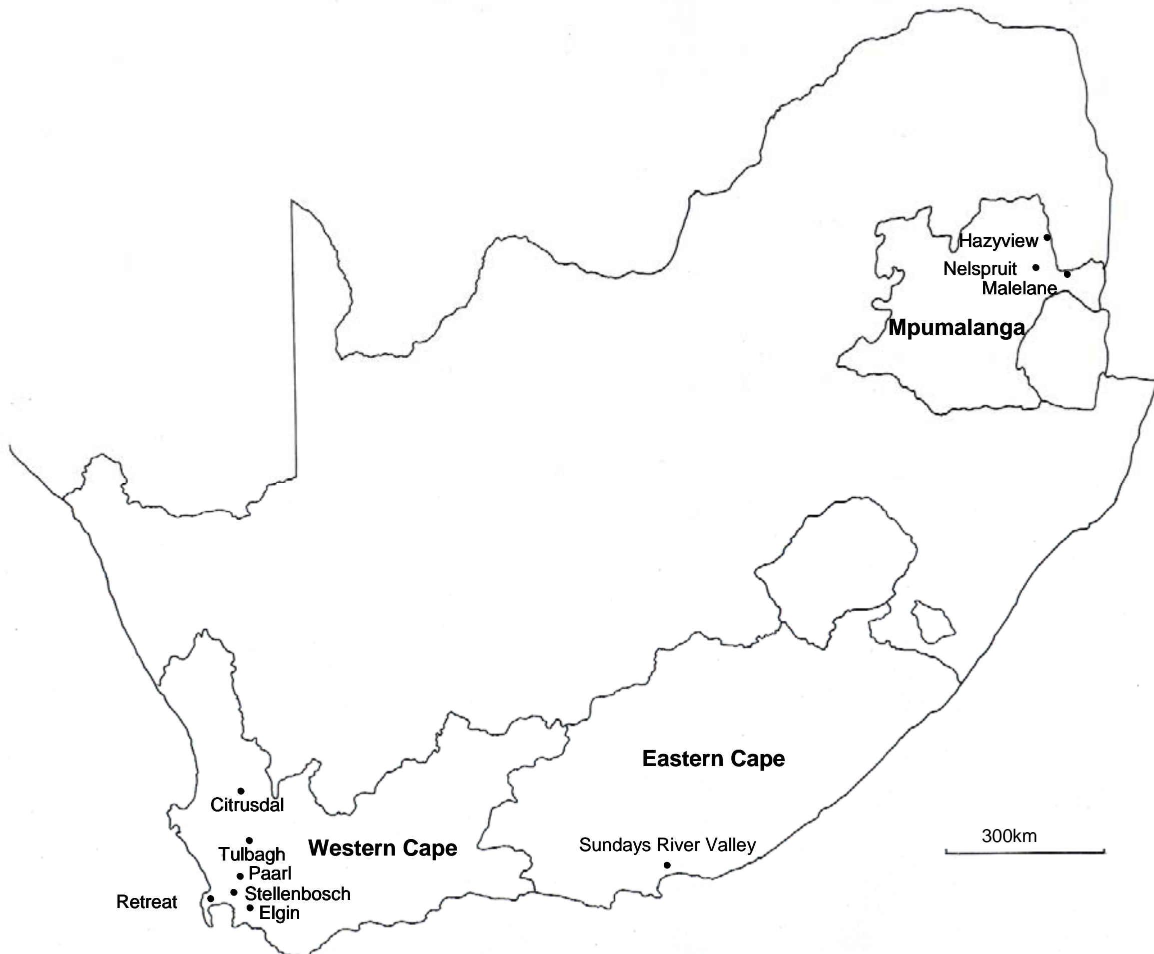


Fig. 9.2. Cluster analysis (UPGMA), based on analysis of 322 AFLP fragments, showing the relationships between *T. leucotreta* populations sampled from three different provinces in South Africa, using a laboratory colony maintained in Britain as an outgroup.

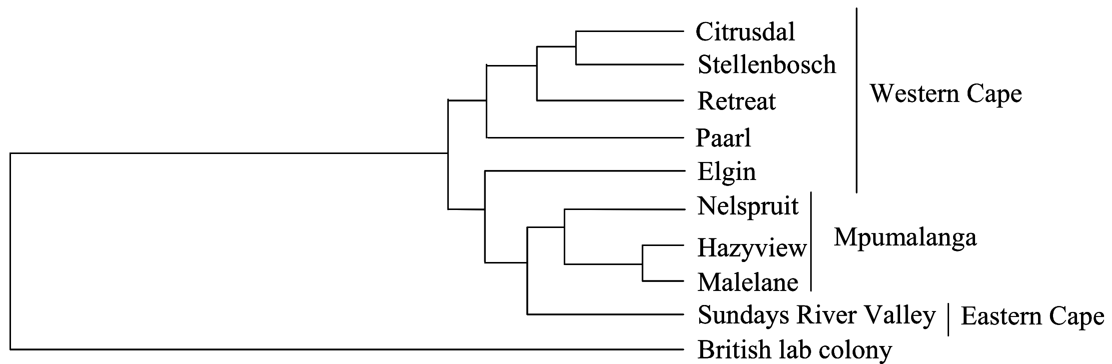
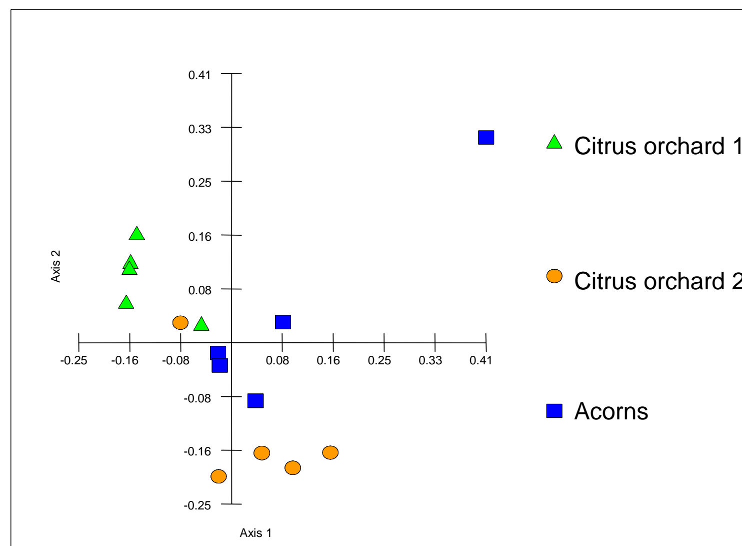


Fig. 9.3. Principal co-ordinate analysis showing the relationships, based on AFLP analysis, between *T. leucotreta* individuals collected from acorns and two citrus orchards situated within 500m of each other in Citrusdal, Western Cape province, South Africa. Axes 1 and 2 account for 25.2% of the variation.



CHAPTER 10

Factors affecting the population genetic structure of six economically important Tortricidae in South Africa, with special reference to carnation worm *Epichoristodes acerbella*

10.1 Abstract

Analysis of the genetic structure of an insect pest may benefit pest management programs by providing greater insight into various ecological aspects. Therefore, the population genetic structure of the Tortricidae of major economic importance in South African agricultural orchards was determined. This study reports on the genetic structure of the carnation worm *Epichoristodes acerbella* and compares these results with those produced previously for *Cydia pomonella*, *Grapholita molesta*, *Thaumatotibia leucotreta*, *T. batrachopa* and *Cryptophlebia peltastica* based on AFLP analysis. *Epichoristodes acerbella* is native to South Africa and polyphagous in the Western Cape region, causing extensive damage to cultivated crops such as grapes, pears and *Protea* spp. Genetic variation within and between seven geographic populations from the Western Cape was determined based on a total of 221 AFLP fragments generated using five selective primer pairs. Extensive genetic divergence was found between *E. acerbella* populations sampled from local spatial scales. Genetic diversity averaging over all loci for all seven populations was found to be high (H = 0.1701) and a significant amount of population differentiation was found (G_{st} = 0.2625). These results are similar to those produced for the other economically important closely related tortricid species in South Africa, which show close similarities in biology and ecology in agricultural systems. However, since the species differ in terms of history (native or introduced) and host range (oligophagous or polyphagous), the effects of these factors on population genetic structure were determined by comparison of species group genetic parameters. No significant differences were found between genetic diversity and genetic differentiation for native and introduced or oligophagous and polyphagous species. It was therefore concluded that factors such as limited dispersal may have played a larger role in shaping the population genetic structure of the tortricids of economic importance in South Africa. These results may be useful for the design of effective pest management programs.

10.2 Introduction

Analysis of population genetic structure may provide insight into aspects that are essential for understanding the ecology of insect agricultural pests. The importance of considering population genetic

parameters when designing pest management programs is continually being realized (Claridge 1996, Loxdale & Lushai 2001). Therefore, a study was undertaken to determine the amount and distribution of genetic variation in tortricid species of major economic importance in South Africa. The population genetic structure, based on amplified fragment length polymorphism (AFLP) analysis, of five tortricid species in South Africa has been determined, namely oriental fruit moth *Grapholita molesta* (Busck) (Chapter 6), codling moth *Cydia pomonella* (L.) (Chapter 7), macadamia nut borer *Thaumatotibia batrachopa* (Meyrick) (Chapter 8), litchi moth *Cryptophlebia peltastica* (Meyrick) (Chapter 8) and false codling moth *T. leucotreta* (Meyrick) (Chapter 9). This investigation reports on the population genetic structure of the final tortricid species of major economic importance in South Africa, namely the carnation worm *E. acerbella* (Walker) (Lepidoptera: Tortricidae).

Epichoristodes acerbella is one of the most important lepidopteran species of economic and quarantine importance in South Africa and elsewhere. The species originates from South Africa (Myburgh & Basson 1961, Oliver & Bolton 1974, Sola 1974, Bolton 1979) and emerged as a pest in the Western Cape region when chemical control against pests such as codling moth proved injurious to its parasite complex (Basson & Myburgh 1960, Myburgh & Basson 1961). Although *E. acerbella* is known mainly as a pest of carnation in Europe, where it was accidentally introduced into various countries (van der Vrie 1991), in South Africa and particularly the Western Cape region, *E. acerbella* is polyphagous. Larval hosts include cultivated fruit such as pome fruit, stone fruit and grapes, ornamental plants such as carnations and *Leucadendron* spp. and various weed species and it is common for larvae to move between hosts (Basson 1960, Myburgh & Basson 1961, Annecke & Moran 1982, Wright 1995). *Epichoristodes acerbella* eggs are laid on the surface of the substrate and larvae roll leaves or bore into fruit or stems, where their development is completed (Oosthuizen 1936, Basson & Myburgh 1960, Myburgh & Basson 1961, Blomefield & du Plessis 2000). Due to its quarantine status, even low levels of infestations by *E. acerbella* can lead to rejections of fruit destined for export and efficient control practices are required to suppress population levels (Deciduous Fruit Grower 1997). However, chemical control of *E. acerbella* is seldom effective due to the inaccessibility of the larvae and cultural practices such as weed removal, although playing a role in decreasing population levels (Blomefield & du Plessis 2000), cannot provide the level of control necessary. For effective use of alternative control practices and more efficient use of existing practices, analysis of population genetic structure and the insight that it can provide into the ecology of *E. acerbella* may be useful.

Ecological studies may benefit the understanding of pest management strategies by identifying key events in insect life histories that influence factors such as the evolution of insecticide resistance and the conditions under which resistance poses problems for pest control (Daly 1994). These include factors such as dispersal rate and host preference, which are implicit in the management of insect population levels

since most strategies are designed to manipulate mortality factors or to enhance the effects of immigration (May & Dobson 1986, Roush & Croft 1986). The factors shaping the population genetic structure of an insect may therefore provide valuable insight into the ecology of the species. Since it is often difficult to distinguish the relative importance of contributing factors that may affect population structure, the effect of various factors on genetic structure may be investigated indirectly by comparative studies of closely related species that differ with regard to only one or two of these factors and testing for correlations. The economically important tortricids in South Africa fit these criteria since they are closely related and show similarities in their biology and ecology. The larvae of all six species are fruit borers and therefore occur in a relatively protected environment; they are all found in agricultural ecosystems and subjected to the pressures that accompany this such as exposure to insecticides. The six tortricid species differ only in the histories of their populations in South Africa (whether native or introduced) and host range (whether oligophagous or polyphagous). Therefore, it is possible to assess the relative effects of these two factors on their population genetic structure.

The objectives of this study were firstly to determine the population genetic structure of *E. acerbella* in the Western Cape and secondly to use this information and that of the five other economically important tortricids in South African orchards to determine the effect of population history and host range on their population genetic structure.

10.3 Material and Methods

10.3.1 Population genetic structure of *E. acerbella* populations

10.3.1.1 Insect material

Specimens were collected from Elgin (August 2004, November 2004), the Hex River Valley (February 2004, August 2004), Jonkershoek (February 2004), Stellenbosch (June-July 2004), Tulbagh (November 2004), the Witzenberg Valley (October 2004) and Worcester (February 2004) in the Western Cape province, South Africa (Table 10.1, Fig. 10.1) from commercial plantings of vineyards, pears, *Protea* spp. and *Leucadendron* spp. To assess variation within regions, populations were sampled from three farms in Stellenbosch and nine farms distributed throughout the Hex River Valley, with the maximum distance between populations = 20 km. Variation over local scales was assessed by sampling populations from orchards situated within one kilometre of each other from the Hex River Valley, Stellenbosch, Tulbagh and the Witzenberg Valley. A total of 113 *E. acerbella* specimens were included for analysis using amplified

fragment length polymorphism (AFLP). Voucher material of specimens included for analysis was deposited in the museum of the Department of Entomology, University of Stellenbosch.

10.3.1.2 DNA analysis

Genomic DNA was extracted from the heads and legs of moths using the CTAB protocol described by Reineke *et al*. (1998). AFLP analysis was performed as originally described (Vos *et. al* 1995), with minor modifications. Genomic DNA (200ng) was digested with five units each of the restriction enzymes *Eco*RI (Promega) and *Mse*I (New England Biolabs). Preselective reactions were performed with oligonucleotides containing no selective nucleotides (*Eco*RI 5'- GAC TGC GTA CCA ATT C-3', *Mse*I 5'- GAT GAG TCC TGA GTA A-3') whereas primers used for selective amplifications each contained five selective nucleotides (*Eco*RI-ATC *Mse*I-CAA, *Eco*RI-TCA *Mse*I-GCA, *Eco*RI-GAC *Mse*I-CTT, *Eco*RI-AGT *Mse*I-CTA, *Eco*RI-GAC, *Mse*I-GCA). Selective amplification products were electrophoresed on 6% (w/v) denaturing polyacrylamide gels at 60 W for 2 - 3 hours. Gels were dried on Whatmann paper and exposed to Kodak Biomax X-ray films for visualization.

10.3.1.3 Data analysis

AFLP fragments were scored and recorded into a binary data matrix with "1" indicating fragment presence and "0" indicating fragment absence. Standard population genetic statistics were calculated with POPGENE version 1.31 population genetics software (Yeh & Yang 1997). Genetic diversity was estimated by determining the average gene diversity over all AFLP loci (*H*) (Nei 1987) and measures of population differentiation calculated using G_{st} co-efficients. Genetic relationships between populations were determined using Nei's (1978) unbiased estimates of genetic identity (*I*) and genetic distance (*D*) based on the frequency of the presence of each band for all primer combinations. These measures of genetic distance were used to construct a dendrogram using the unweighted pair-group method with an arithmetic mean (UPGMA). The software MVSP Version 3.11c (Kovach 1999) was used to view the relationships between individuals using Q-mode principal co-ordinate analysis (PCOA) based on dissimilarity matrices generated using Gower's general similarity co-efficient and UPGMA cluster analysis based on the Jaccard co-efficient (Sneath & Sokal 1963). Analysis of molecular variance (AMOVA) was performed to determine the distribution of genetic variability within and among populations using the software GenAlEx version 6 (Peakall & Smouse 2005), with the number of permutations set at 1000.

10.3.2 Comparison between species

The amount and distribution of genetic variation was compared between the six species. The genetic parameters G_{st} and *H* were compared using a Chi squared test to determine whether these differed significantly. To determine whether these parameters differed between introduced and native species and oligophagous and polyphagous species, single factor ANOVA analysis was used. Species were classified as oligophagous if the larvae were able to feed on a maximum of two or three host species or polyphagous if a minimum of four host species were utilized.

10.4 Results

10.4.1 Population genetic structure of *E. acerbella* populations

10.4.1.1 Genetic diversity

AFLP analysis of 113 *E. acerbella* individuals using five primer combinations yielded a total of 221 fragments for analysis. Genetic diversity within the total *E. acerbella* population in the Western Cape was calculated as $H = 0.1701$, with 95.93% of the fragments polymorphic at the 95% level. Within regions, genetic diversity indices ranged between $H = 0.0273$ ($p = 29.4\%$) in Jonkershoek populations to $H = 0.1656$ ($p = 91.19\%$) in Tulbagh (Table 10.2).

10.4.1.2 Genetic differentiation

Among regions. Population differentiation estimates of $G_{st} = 0.2625$ were calculated for *E. acerbella* populations from seven geographic regions in the Western Cape. Estimates of genetic distance (Nei 1978) ranged between $D = 0.1228$ ($I = 0.8845$) between Jonkershoek and Tulbagh populations and $D = 0.0074$ ($I = 0.9926$) between Stellenbosch and Hex River Valley populations (Table 10.3). The relationships between *E. acerbella* populations were viewed using UPGMA analysis (Fig. 10.2). Cluster analysis indicated a major division between the Jonkershoek population and all others. The Stellenbosch and Hex River Valley populations and the Elgin and Tulbagh populations appeared to be closely related, with the Worcester and Tulbagh populations more distantly related to these.

Within regions. Variation within regions was assessed by analyzing populations sampled from three sites in Stellenbosch and nine farms distributed throughout the Hex River Valley. In both Stellenbosch and Hex River Valley populations, individuals that were collected from the same site/farm appeared to be closely related using cluster analysis and it was possible to more or less ascribe individuals to populations based on their AFLP profiles. Measures of population differentiation were calculated as $G_{st} = 0.3847$ for Stellenbosch populations and $G_{st} = 0.4347$ for Hex River Valley populations.

Local geographic scales. Variation over local scales was assessed by sampling populations from orchards situated within one kilometre of each other from the Hex River Valley, Stellenbosch, Tulbagh and the Witzenberg Valley. For all populations, a significantly high proportion of the variability could be attributed to variation within populations (Table 10.4). Variation among orchards was also significant ($P < 0.05$) and varied from 17% (Stellenbosch) to 34% (Tulbagh). In the total population, 31% of the variation was ascribed to variation among populations. It was possible to discriminate among populations sampled from orchards situated within one kilometre of each other using cluster analysis. An example is shown in Fig. 10.3.

10.4.2 Comparison between species

The origin and host status for each of the six tortricid species are shown in Table 10.5, along with estimates of genetic differentiation G_{st} and genetic diversity H. These parameters did not differ significantly at the species level when analyzed using a Chi squared test ($P > 0.05$). Statistical differences between G_{st} and H values between native and introduced as well as oligophagous and polyphagous species were investigated using single factor ANOVA analysis. However, estimates of population differentiation (G_{st}) and genetic diversity (H) did not differ significantly when tested against population history ($P = 0.868$ and 0.980 respectively) and host range ($P = 0.540$ and 0.274 respectively).

10.5 Discussion

10.5.1 Population genetic structure of *E. acerbella* populations

Genetic diversity between *E. acerbella* populations in the Western Cape was high and at population level AFLP analysis detected clear variation between populations sampled from seven regions. There appeared to be no geographic pattern in the relationships between populations, which may partly be due to

agricultural practices such as the movement of fruit or nursery material. The latter may be evident in the close relationship between populations sampled from the Hex River Valley and Stellenbosch, as both regions are known for intensive wine grape production, which may have facilitated the exchange of plant material between regions. The major division between the Jonkershoek population and all others may be related to the fact that this population was collected from a nature reserve whereas all other populations were collected in agricultural systems. These results may be due to the differences in habitats between the populations (for example control practices such as pesticide exposure and availability of alternative larval resources). At local scales genetically distinct populations were evident and extensive divergence was found, most likely indicating that gene flow between *E. acerbella* populations in Western Cape orchards is limited. These results may have important implications for managing *E. acerbella* population levels and may indicate that practices that are more effective when gene flow is low, such as pheromone mating disruption, may prove useful for managing *E. acerbella* population levels in Western Cape orchards. This study provides the first account of the population genetic structure of *E. acerbella* populations. Knowledge of the population genetic structure of *E. acerbella* in its country of origin may improve understanding of the ecology of the species in the European countries where it has become established. This information may also aid the design of eradication campaigns should the species become established as an exotic invasive pest, which is a serious threat since *E. acerbella* continues to be intercepted in countries such as England (Allen 1980) and the USA (Deciduous Fruit Grower 1997).

The population genetic structure of *E. acerbella* may be affected by a variety of factors. By comparison of population genetic parameters of this species with those of *C. pomonella*, *G. molesta*, *T. leucotreta*, *T. batrachopa* and *Cr. peltastica* insight into these factors was obtained.

10.5.2 Comparison between species

No significant differences were found between the amount and distribution of genetic variation between populations of *C. pomonella*, *G. molesta*, *T. leucotreta*, *T. batrachopa*, *Cr. peltastica* and *E. acerbella*. For all six species high levels of variation were found between populations and extensive divergence over local spatial scales was evident. Since the species differed in terms of population history and host range, the effect of these factors on population genetic structure was investigated.

10.5.2.1 Host range

The host range of a species may affect the partitioning of genetic variation by restricting populations to the area where the host plants occur. The host ranges of the tortricid species investigated may be closely

linked to host plant availability. Hosts should be more readily available for the polyphagous species *T. leucotreta*, *Cr. peltastica* and *E. acerbella* since these are able to feed on a variety of weeds and other non-cultivated hosts and their hosts are found abundantly in both agricultural and natural systems. In contrast, the hosts of the oligophagous species *G. molesta*, *C. pomonella* and *T. batrachopa* are limited mainly to agricultural ecosystems and are therefore relatively scarce or geographically distant compared to those of the polyphagous species. Therefore, it may be expected that the relative scarcity of hosts for *C. pomonella*, *G. molesta* and *T. batrachopa* may have resulted in greater levels of population subdivision than in *T. leucotreta*, *Cr. peltastica* and *E. acerbella*, since no evidence was obtained to suggest that host specific strains occur in any of the species. This hypothesis was examined by determining the correlation between the host range of species with the genetic parameters G_{st} and *H* between oligophagous and polyphagous species. However, neither parameter differed significantly between the oligophagous and polyphagous species. Therefore, it may appear as if factors other than host range provided a greater contribution towards shaping the population genetic structure of these economically important Tortricidae in South Africa.

10.5.2.2 Historical effects

The consequences of historical effects on population genetic structure are well known (Lowe *et al.* 2004). The relative contribution of historical effects on shaping population genetic structure may be variable and in some cases population genetic estimates may contain information more relevant to historical patterns of gene flow than to the current population dynamics (Larson *et al.* 1984, Liebherr 1988, Avise 1992, Bossart & Prowell 1998). The effect of population history on genetic structure was investigated indirectly by comparative studies of the tortricids studied and testing for a correlation between the genetic parameters of native and introduced species. Theoretically, the introduced species should show lower levels of diversity than the native species due to population bottlenecks as a result of founder effects (Lowe *et al*. 2004). The consequences of historical effects on the population genetic structure of the economically important Tortricidae was investigated by comparing levels of the genetic parameters G_{st} and *H* between species that were introduced from elsewhere (*C. pomonella* and *G. molesta*) with those that are native to South Africa (*T. leucotreta*, *T. batrachopa*, *Cr. peltastica* and *E. acerbella*). However, no statistical difference could be found between these genetic parameters for the introduced and native species. Therefore, it is likely that factors other than population history were more important in shaping population structure in the economically important Tortricidae in South Africa and that other effects have played a greater role in differentiating between populations.

Mopper (1996) identified two factors besides population history and host range that may give rise to population differentiation in phytophagous insects, namely dispersal and geographic barriers. In agricultural systems, control practices and human-mediated activities such as the movement of infested

fruit between regions may also play a role in counteracting the effects of gene flow and producing genetic structure at local spatial scales. Each of these factors can be invoked to account for the genetic structure of the six tortricid species studied and has been considered in earlier chapters (Chapters 6-9). In particular, the limited dispersal of the six species, confirmed by mark-recapture or flight-mill studies for *C. pomonella*, *G. molesta* and *T. leucotreta* (Mani & Wildbolz 1977, Sziraki 1979, Vickers *et al*. 1985, Rothschild & Vickers 1991, Schumacher 1997, Newton 1998, Keil 2001), appears to have played a major role in partitioning genetic variation. These results are useful for understanding the ecology of the tortricid species of economic importance in South Africa, facilitating, in turn, the improvement of their eventual control.

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Tables

Table 10.1. Geographic location of *E. acerbella* specimens from South Africa used for AFLP analysis.

Geographic location	Farm name	Latitude	Longitude	Orchard codes*
Elgin	Elgin Experimental Farm	34°9'S	19°2'E	-
	Oak Valley	34°9'S	19°3'E	-
Jonkershoek	-	33°59'S	18°57'E	-
Stellenbosch	Timberlea	33°55'S	18°52'E	R44, Back Hill, 10
	Devon Valley	18°50'S	33°54'E	*Protea* block, *Leucadendron* block, grape block
	(Onderpapegaaiberg)	33°56'S	18°52'E	-
Hex River Valley	Bella Vista	33°28'S	19°40'E	-
	Boplaas	33°30'S	19°36'E	B5, B6
	Cairngorm	33°30'S	19°32'E	-
	De Vlei	33°20'S	19°41'E	9, 10
	Idlewinds	33°30'S	19°34'E	-
	Kanetvlei	33°31'S	19°32'E	-
	Klipheuwel	33°30'S	19°31'E	B03, A04
	Moreson	33°28'S	19°37'E	-
	Ruimsig	33°29'S	19°37'E	-
	Somerlus	33°28'S	19°38'E	-
	Tesame	33°27'S	19°39'E	-
Tulbagh	Roodezand	33°15'S	19°7'E	-
	Twee Jonge Gezellen	33°15'S	19°7'E	Front pears, back pears
Witzenberg Valley	Paardekloof	19°15'S	33°15'E	a, b, KW2B, KO7
Worcester	Protea	33°36'S	19°24'E	-

* if samples were analyzed from multiple orchards

Table 10.2. Genetic diversity estimates for *E. acerbella* populations, based on AFLP analysis, sampled from seven different regions in the Western Cape.

	p (%)	*H*
Jonkershoek	29.4	0.0273
Stellenbosch	95.70	0.1528
Hex River Valley	97.56	0.1575
Worcester	75.00	0.0839
Elgin	89.15	0.1491
Tulbagh	91.19	0.1656
Witzenberg Valley	90.45	0.1525
Population means	*81.21*	*0.1270*
Total	95.93	0.1701

p (%) percent polymorphic loci, *H* Nei's gene diversity,

Table 10.3. Estimates of genetic distance and genetic identity between *E. acerbella* populations sampled from seven regions in the Western Cape, based on analysis of 221 AFLP fragments.

	Jonkershoek	Stellenbosch	Hex River Valley	Worcester	Elgin	Tulbagh	Witzenberg Valley
Jonkershoek	-	0.9097	0.9048	0.9064	0.8962	0.8845	0.9064
Stellenbosch	0.0947	-	0.9926	0.9706	0.9754	0.9551	0.9762
Hex River Valley	0.1000	0.0074	-	0.9731	0.9758	0.9521	0.9758
Worcester	0.0982	0.0298	0.0272	-	0.9615	0.9396	0.9621
Elgin	0.1095	0.0249	0.0245	0.0393	-	0.9574	0.9820
Tulbagh	0.1228	0.0459	0.0491	0.0623	0.0436	-	0.9765
Witzenberg Valley	0.0982	0.0240	0.0245	0.0387	0.0182	0.0238	-

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 10.4. Analysis of molecular variance (AMOVA), based on AFLP analysis, for *E. acerbella* populations sampled from orchards situated within one kilometre of each other from the Western Cape province, South Africa.

	df	SS	MS	Est. Var.	Total Variance (%)	*P*
Among populations						
Hex River Valley	1	52.500	52.500	8.208	29	<0.05
Stellenbosch (Devon Valley)	2	92.621	46.311	5.981	24	<0.001
Stellenbosch (Timberlea)	2	57.050	28.525	3.479	17	<0.05
Tulbagh	1	53.262	53.262	9.925	34	<0.05
Witzenberg Valley	3	118.083	39.361	6.075	25	<0.001
Total	13	641.943	49.380	8.178	31	<0.001
Within populations						
Hex River Valley	6	118.00	19.667	19.667	71	<0.05
Stellenbosch (Devon Valley)	11	203.950	18.541	18.541	76	<0.001
Stellenbosch (Timberlea)	7	121.750	17.393	17.393	83	<0.05
Tulbagh	5	96.167	19.233	19.233	66	<0.05
Witzenberg Valley	10	182.417	18.242	18.242	75	<0.001
Total	39	722.283	18.520	18.520	69	<0.001

Table 10.5. Comparative population genetic parameters for six economically important species sampled from South Africa, based on AFLP analysis.

	Origin	Host range	G_{st}	*H*
G. molesta	Introduced	Oligophagous	0.2790	0.1820
C. pomonella	Introduced	Oligophagous	0.3778	0.1797
T. leucotreta	Native	Polyphagous	0.2929	0.1490
T. batrachopa	Native	Oligophagous	0.3580	0.2219
Cr. peltastica	Native	Polyphagous	0.3704	0.1909
E. acerbella	Native	Polyphagous	0.2625	0.1701

Figures

Fig. 10.1. Map of the Western Cape showing the geographic locations of the seven regions from which *E. acerbella* populations were sampled for analysis of population genetic structure.

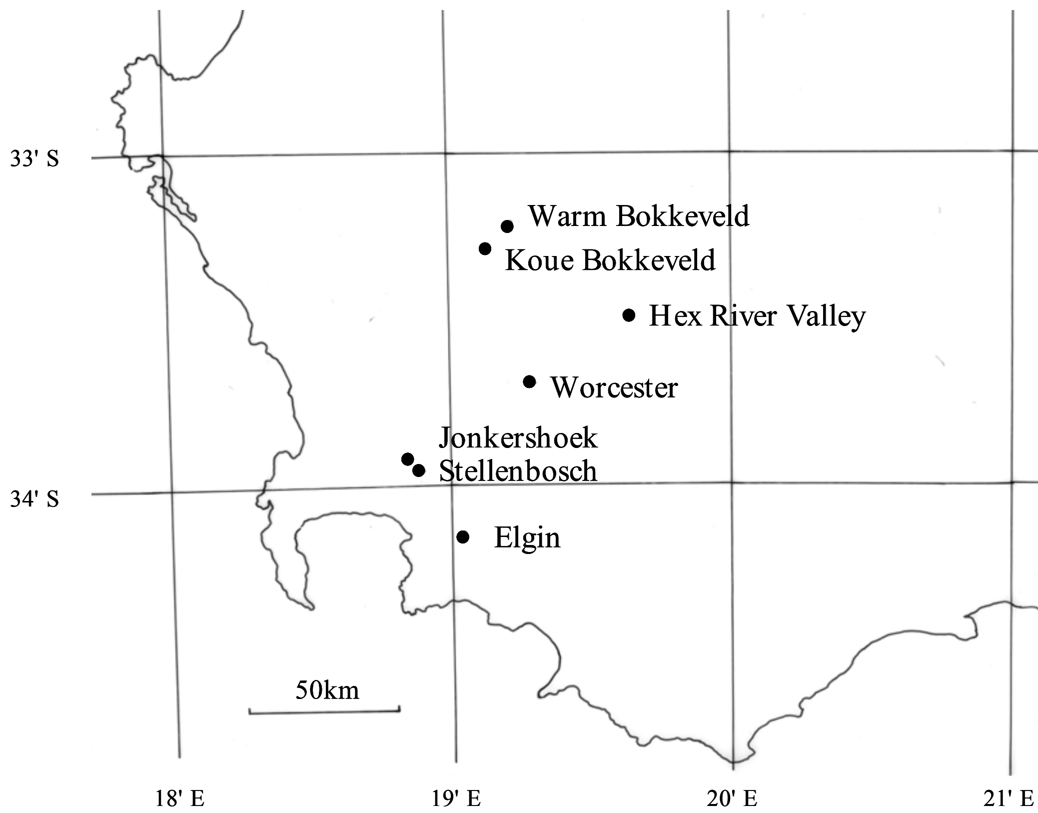


Fig. 10.2. Cluster analysis (UPGMA) showing the relationships between *E. acerbella* populations sampled from seven regions in the Western Cape, based on AFLP analysis.

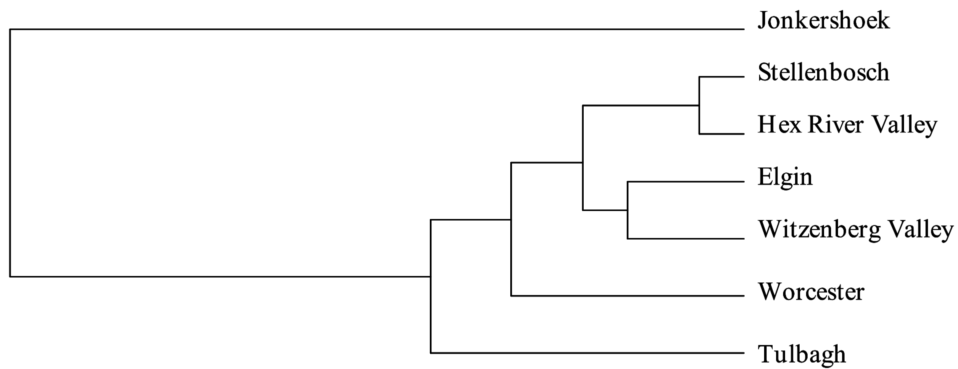
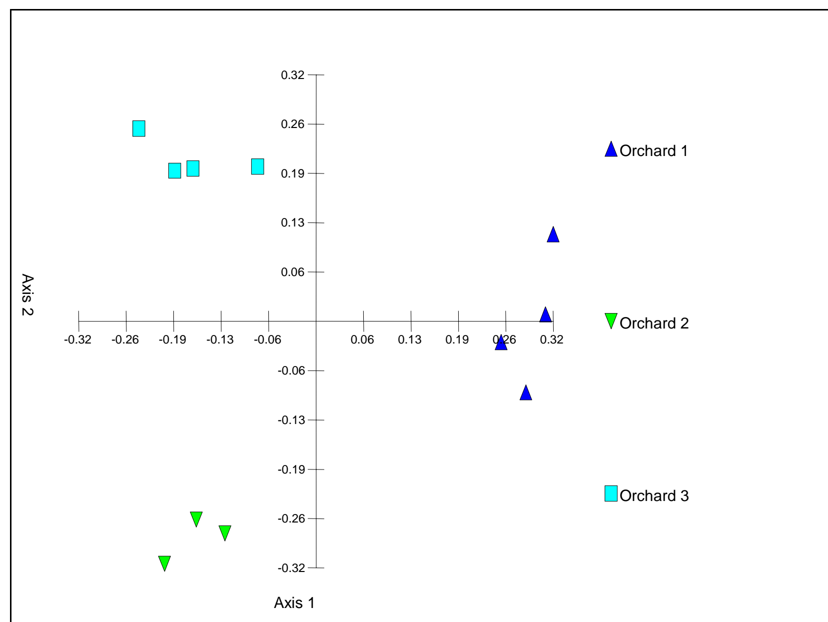


Fig. 10.3. Principal co-ordinate analysis showing the relationships between *E. acerbella* individuals sampled from three orchards situated within one kilometre of each other from Tulbagh, South Africa, based on AFLP analysis.



CHAPTER 11

CONCLUSION

The aim of this study was to contribute towards the control of those Tortricidae of major economic importance to the South African fruit industry, namely the false codling moth *Thaumatotibia leucotreta*, the macadamia nut borer *T. batrachopa*, the litchi moth *Cryptophlebia peltastica*, the carnation worm *Epichoristodes acerbella*, the oriental fruit moth *Grapholita molesta* and the codling moth *Cydia pomonella*. Specifically, it was aimed, firstly, to provide a morphological means of identifying and discriminating between the relevant immature stages of these six species and, secondly, to analyze the amount and distribution of genetic variation between their metapopulations. The accomplishment of these aims is outlined below.

1. Identification of immature stages

The identification of any insect life stage requires an accurate description of its morphology. The larvae and pupae of the introduced species *C. pomonella* and *G. molesta* and the Afrotropical *T. leucotreta* have been described previously, but descriptions of the Afrotropical *T. batrachopa*, *Cr. peltastica* and *E. acerbella* are lacking. Therefore, this study examined and described, with illustrations, the morphology of the final instar larvae and pupae of the four Afrotropical tortricid species of major economic importance in South Africa (Chapters 2-5). This was compared with the morphology of the final instar larvae and pupae of *C. pomonella* and *G. molesta*. Keys based on morphological characters were constructed to distinguish between the species feeding on tropical and subtropical fruit (Chapters 2 and 4) and deciduous fruit (Chapters 3 and 5). These descriptions and keys should facilitate unambiguous identification of the final instar larvae and pupae of the economically important Tortricidae in South Africa and should be of valuable use for quarantine and pest control officers. At present, identifying earlier larval instars may still be problematic and further research is necessary to examine methods of identifying these stages using either morphological or molecular means.

2. Population genetic structure

The population genetic structure of each of the six tortricid species of major economic importance in South Africa was characterised using AFLP analysis (Chapters 6-10). This study represented the first instance of the determination of the population genetic structure of *G. molesta*, *T. batrachopa*, *Cr. peltastica*, *T. leucotreta* and *E. acerbella* in agricultural systems and the first time that the population genetic structure of *C. pomonella* was determined in South African orchards. Results indicated that in populations of all six

species, population genetic variation was high and populations were genetically differentiated over both large and local geographic scales (Chapter 10). These results were interpreted in terms of the field behaviour of the pests and it was suggested that limited dispersal was one of the primary factors influencing the population genetic structure of the six tortricid species. Insect dispersal affects pest control practices such as the management of insecticide resistance, pheromone mating disruption and the sterile insect technique (SIT). These practices rely on an areawide approach and attempt to address the problem within the appropriate ecological context, thus requiring an understanding of pest dispersal at different spatial scales. This study determined that dispersal of the aforementioned tortricids among orchards was limited, indicating the appropriate management scale for effective application of pheromone mating disruption and SIT. In addition, findings indicate that highly localised levels of insecticide resistance may be expected in each of the species, which should be taken into account when designing insecticide resistance management programs. The results of this study are therefore beneficial for the development of successful pest management strategies.

In conclusion, both aims of the study were successfully met. The identification of the immature stages and analysis of population genetic variation should both contribute towards more effective control of the tortricid species of major economic importance in South Africa and facilitate the export potential of South African fruit.