Environmental physiology of *Eldana saccharina* (*Lepidoptera: Pyralidae)* in South Africa: implications for pest management

by
Elizabeth Kleynhans

Dissertation presented for the degree of Doctor of Philosophy in the Faculty of AgriSciences at Stellenbosch University

Supervisor: Prof. Desmond E. Conlong
South African Sugar Association Experiment Station, Mount Edgecombe, Durban, South Africa; Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa

Co-supervisor: Prof. John S. Terblanche
Centre for Invasion Biology, Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Stellenbosch, South Africa

December 2014

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2014

Abstract

Eldana saccharina Walker (Lepidoptera: Pyralidae) is a stem borer and food crop pest of economic importance. Temperature and moisture availability possibly influence *E. saccharina* distribution and abundance, however, the thermal biology and desiccation physiology of *E. saccharina* are not fully understood. Furthermore, physiological adaptation probably facilitates the invasion success of *E. saccharina* into novel environments and this too remains unstudied. Here, the thermal- and desiccation-trait variation of *E. saccharina* were studied and population responses were modelled. The results of this work provided insights into novel physiological outcomes of *E. saccharina* that is coupled with its environmental climatic stress resistance, overwintering ability and population fitness in general. In determining thermal limits to activity and survival of *E. saccharina* results showed that chill coma onset temperature (CT_{min}) and critical maximum temperature (CT_{max}) of *E. saccharina* moths collected from sugarcane (*Saccharum* spp. hybrids) were significantly lower than those from *Cyperus papyrus* L. (CT_{min} = 2.8 ± 0.4 *vs*. 3.9 ± 0.4 °C; CT_{max} = 44.6 ± 0.1 *vs*. 44.9 ± 0.2 °C, $P < 0.0001$ in both cases). These results holds important implications for habitat management (or 'push-pull') strategies in the sense that host plant may strongly mediate lower critical thermal limits. Results for pronounced variation in adult CT_{min} (± 4 °C) across the geographic range of *E. saccharina* in South Africa was found and it was significantly positively correlated with the climatic mean minimum temperature. Slower developmental time in the most low-temperature tolerant population suggests lower CT_{min} adaptation has come at a cost to fitness, but allows greater survival and activity in that environment. There are a significant reduction of phenotypic plasticity in the laboratory population and a strong genetic component to CT_{min} trait variation. Physiological acclimation within a single generation, during immature life stages, resulted in altered adult water balance physiology to enhance fitness. Results from a biophysical population model showed that over-wintering life stage and climate significantly affected the number of *E. saccharina*

generations, predicted stress, relative moth fitness and relative adult abundance. Larval over-wintering led to less generations and more frequent cold- and heat stress at a cold field site compared to a warm one. This in turn reflected on the relative adult fitness and –abundance. Larval presence predictions overlapped well with positive scout records averaged across a matrix of sugarcane ages and cultivars. The results from this work are important on which to base integrated pest management strategies and are applicable to a large audience across agricultural landscapes and in the sugarcane industry of South Africa.

Opsomming

Eldana saccharina Walker (Lepidoptera: Pyralidae) is ‘n stamboorder en voedselgewas pes van ekonomiese belang. Alhoewel ons nie die termiese biologie en uitdrogings-fisiologie van *E. saccharina* verstaan nie, beïnvloed temperatuur en lugvog inhoud waarskynlik die verspreiding en teenwoordigheid van *E. saccharina*. Fisiologiese aanpassing fasiliteer waarskynlik die indringing sukses van *E. saccharina* in nuwe omgewings en navorsing op hierdie verskynsel is ook nog nie gedoen nie. Die variasie in termiese- en uitdrogingseienskappe van *E. saccharina* is hier genavors en populasie reaksies is gemodeleer. Die resultate van hierdie werk gee insig tot nuwe fisiologiese uitkomste van *E. saccharina* wat gekoppel is met omgewings-klimaat stres weerstand, oorwintering vermoë en die algemene populasie geskikdheid. Die termiese limute vir aktiwiteit en oorlewing van *E. saccharina* resultate het getoon dat temperature waar koue-koma opgemerk word (CT_{min}) en kritieke maksimum temperature (CT_{max}) van *E. saccharina* motte wat uit suikerriet (*Saccharum* spp. hibriede) versamel is, betekenisvol laer is in vergelyking met díe gemeet vir motte uit *Cyperus papyrus* L. versamel (CT_{min} = 2.8 ± 0.4 *vs*. 3.9 ± 0.4 °C; CT_{max} = 44.6 ± 0.1 *vs*. 44.9 ± 0.2 °C, $P <$ 0.0001 in albei gevalle). Hierdie resultate het belangrike gevolge vir habitat (of ‘push-pull’) strategië in die sin dat gasheerplant lei tot veranderede kritieke temperatuur-limute van *E. saccharina*. Daar is betekenisvolle variasie in volwasse mot CT_{min} (± 4 °C) oor die geografiese verspreiding van *E. saccharina* in Suid-Afrika en dit is betekenisvol positief gekorreleerd met die gemiddelde minimum temperatuur. ‘n Stadige ontwikkelingstyd in die mees koue-tolerante populasie opper dat ‘n laer CT_{min} aanpassing tot ‘n fiksheidskoste lei, alhoewel dit aktiwiteit en oorlewing in die omgewing verbeter. Daar is ‘n betekenisvolle afname van fenotiepe-plastisiteit in die laboratorium kolonie en ‘n sterk genetiese komponent aan die variasie in die CT_{min} eienskap gekoppel. Fisiologiese akklimasie binne ‘n enkele generasie, deur die onvolwasse lewensstadia, het gelei tot veranderde water balans fisiologie om die geskikdheid van volwasse motte te verbeter.

Resultate van die biofisiese populasie model het getoon dat die oorwinteringstadium en ook klimaat ‘n betekenisvolle effek het op die aantal generasies, voorspelde stres, relatiewe-geskikdheid en -voorkoms van *E. saccharina*. Larwale oorwintering het gelei tot minder generasies en meer dikwelse koue- en hitte stres in ‘n kouer gebied in vergelyking met ‘n warmer gebied. Hierdie waarnemings in die model voorspellings het op die volwasse fiksheid en –teenwoordigheid weerspieël. Die voorspelde teenwoordigheid van larwes het goed met positiewe veldopnames oorvleuel oor ‘n matriks van suikerriet ouderdomme en kultivars. Die resultate van hierdie werk is belangrik en moet gebruik word om geïntegreerde plaagbestuur strategië op te basseer. Die resultate is van toepassing op ‘n wye gehoor oor landbou in die algemeen en veral vir die suikerriet industrie van Suid-Afrika.

Acknowledgements

To my mentor and supervisor, John Terblanche, thank you for your credence in my abilities and work. Your unwavered support and guidance will always be appreciated. For all the support and constructive comments, I thank Des Conlong, whom I respect and appreciate dearly. Karlien Trumpelmann, Tom Webster, Andreas Mthethwa, Nelson Muthutsamy, Sphelele Mlumbi and Angela Walton provided logistical support and I left dear friends behind at the South African Sugarcane Research Institute's rearing unit upon completion of my work there. Special thanks go to the team at Eston in the southern Midlands of KwaZulu-Natal for help on obtaining specimens from the field. I want to share my condolences with Mick Hampson's family and friends. I enjoyed, and was inspired by, Mick's enthusiasm and great perspective on life. He will be remembered.

Financial and logistical support was provided by the South African Sugarcane Research Institute. Further supported from HortGro Science, the Centre for Invasion Biology and the National Research Foundation Incentive Funding for Rated researchers contributed indirectly toward this work.

To my family, thank you for motivating me and for supporting my decisions in full. To my friends and colleagues, thank you for your great enthusiasm in and around the workplace. I am also grateful for the constructive comments made by Katherine Mitchell on the first research chapter and her substantial inputs into the second research chapter. I also thank and acknowledge the anonymous referees who reviewed and helped to improve the chapters prior to publication of the work.

Table of contents

ABSTRACT ..... **III**

OPSOMMING ..... **V**

ACKNOWLEDGEMENTS ..... **VII**

LIST OF FIGURES ..... **X**

LIST OF TABLES ..... **XIV**

1. **GENERAL INTRODUCTION TO THE PHYSIOLOGY OF *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE)** **1**

1.1 REFERENCES 6

2. **HOST PLANT-RELATED VARIATION IN THERMAL TOLERANCE OF *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE)** **11**

2.1 INTRODUCTION 12

2.2 MATERIALS AND METHODS 14

2.3 RESULTS 20

2.4 CONCLUSION 26

2.5 REFERENCES 29

3. **EVOLVED VARIATION IN COLD TOLERANCE AMONG POPULATIONS OF *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE) IN SOUTH AFRICA** **35**

3.1 INTRODUCTION 36

3.2 MATERIALS AND METHODS 39

3.3 RESULTS 46

3.4 CONCLUSION 52

3.5 REFERENCES 57

4. **DIRECT AND INDIRECT EFFECTS OF DEVELOPMENT TEMPERATURE ON ADULT WATER BALANCE TRAITS OF *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE) 69**

4.1 INTRODUCTION 70

4.2 MATERIALS AND METHODS 73

4.3 RESULTS 75

4.4 CONCLUSION 81

4.5 REFERENCES 84

5. **LOCAL CLIMATE AFFECTS TEMPORAL POPULATION PERFORMANCE OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE) 93**

5.1 INTRODUCTION 94

5.2 MATERIALS AND METHODS 97

5.3 RESULTS 102

5.4 CONCLUSION 113

5.5 REFERENCES 116

6. **SYNTHESIS AND GENERAL DISCUSSION 125**

6.1 REFERENCES 132

List of figures

Figure 2.1. Host-plant induced variation in (A) *E. saccharina* moth critical thermal minima, (B) moth critical thermal maxima, (C) logistic curve of the lower lethal temperatures of moths originally from *C. papyrus* (black upside down triangles, solid line) or sugarcane (blue upright triangles, stippled line), and (D) puparial freezing (supercooling point) temperatures. Box-plots summarize the median (horizontal line), 25th and 75th percentiles [i.e., location of the middle 50% of the data (bottom and top of the box)] and the minimum – maximum data range [when there are no outliers, else, 1.5 times the interquartile ranges (whiskers)]. Outliers, identified as data points more than 1.5 times the interquartile range, are plotted individually. Notches, drawn as a 'waist' in a triangular shape from both sides of the median give an indication of a significant difference between two medians on a 95% confidence interval (CI) when no overlap occurs between two plots' notches (i.e. triangle height indicates 95% CIs). The asterisks in panels A and B indicate significant differences between host plants ('ns' indicates no significant difference). In panel C, multiple points are plotted as 'sunflowers' with multiple 'leaves' indicating overplotting (R, graphics package).....22

Figure 2.2 To determine whether freezing was coupled with death in *E. saccharina* moths I carried out a supercooling point (SCP) mortality essay. Moths were removed at -10 °C (n = 30; removed before freezing) from a standard 'ramping' treatment (0.1 °C per min from 5.0 °C) and upon observation of the exotherm (n = 27; removed upon freezing). Mortality was scored after 24 h at rearing conditions with water provided *ad libitum*. No moths survived after removal upon freezing. 23

Figure 3.1. A) Geographic collection sites of wild *Eldana saccharina* larvae from sugarcane across a range of thermal habitats in South Africa. B) Differences in chill coma onset temperature (CT_{min},

in °C) between wild geographic lines and laboratory reared moths (left: change in CT_{min} relative to the laboratory population; right: absolute CT_{min} estimates) with means and standard errors (s.e.) plotted. C) Correlations of mean weekly minimum, daily and maximum temperatures with CT_{min} results from four different wild geographic lines.....41

Figure 3.2. Box-plots of variation in critical thermal minima (°C) as a result of A) adult age and B) sex (two-day-old adults). No overlap of triangular box-plot notches were used to identify significant differences in group medians on a 95 % confidence level (Crawley 2007). * Indicates significant differences and NS = non-significant. Preliminary trials were conducted using the laboratory colony to achieve the goals of establishing whether age and sex significantly affected CT_{min} estimates for E. saccharina. One-, two- and three-day-old moths' CT_{min} were measured for males and females separately (N = 30 moths per age and sex, total N = 150). The results of age and sex were analysed and interpreted independently using non-parametric statistics after testing for a significant interaction effect using a generalized linear model (GLZ) with a normal probability distribution of errors and an identity link function. Age data were analysed using a GLZ (specified chi-squared test) and sex data were analysed using the Wilcoxon-Mann-Whitney rank sum test. Age significantly influenced the CT_{min} of *E. saccharina* moths, irrespective of sex (Wald χ^2 (1) = 245.86, $P < 0.0001$) while no significant differences were found between the medians of male and female moth CT_{min} irrespective of their age (W = 3468.50, $P = 0.68$). 48

Figure 3.3. A) CT_{min} responses of two-day old moths from the Midlands south, laboratory and Malelane populations acclimated for 24 hours at 22, 27 or 32 °C Vertical bars denote 95% confidence limits. B) A comparison of the variation in CT_{min} from the parental (F_0: Malelane [warm population with low cold tolerance] and Midlands south [cold population with higher cold tolerance]) populations, their crosses (F_1) and a further F_1 x F_1 cross (F_2). C) CT_{min} estimates of F_0

and F_1 by sex following crosses in both directions i.e. Female Malelane x Male Midlands south and *vice versa*. Vertical bars denote 95% confidence limits. 51

Figure 3.4. A) Mean egg to adult development time and B) pupal survival of the Malelane and Midlands south populations when reared under common-garden conditions. Vertical bars denote 95% confidence limits.....52

Figure 4.1. (A) Initial body water content ($BWC_{INITIAL}$, mg) for male and female adult *E.*. Acclimation outcomes pooled. (B) Means (± 95 % CI) of body mass as a result of sex and developmental temperature (20.0 °C, 25.0 °C or 30.0 °C) are shown. 78

Figure 4.2 (A) Adult moth water loss rate (WLR, mg/h), and (B) time to death (h) at different standard larval acclimation temperatures; and the correlations between water loss rate and body mass (mg) (C) and time to death and body mass (D). Water loss was measured over a 24 h period following developmental acclimation (larva to adult) at 20.0 °C, 25.0 °C or 30.0 °C. The means ± 95 % confidence intervals are shown for the acclimation outcomes and the data are corrected for the continuous predictor: body mass. In the scatterplots different symbols and regression lines represent the three acclimation temperature outcomes. 79

Figure 4.3 (A) Adult moth initial body water content ($BWC_{INITIAL}$, % of body mass) calculated as the difference between dry body mass (mg) and initial body mass (mg), presented as a fraction of initial body mass and (B) critical body water content (BWC_{CRIT}, % of body mass) calculated as the dry body mass minus the body mass at death (mg) presented as a fraction of initial body mass at different larval acclimation temperatures. The means ± 95 % confidence intervals are shown for the acclimation outcomes and the data are corrected for the continuous predictor: body mass. Correlations between body water contents and body mass (C and D) is given with different symbols representing the different acclimation groups.....80

Figure 5.1 The impact of local climate and over-wintering stage on A & B) the number generations completed and C & D) life stage transition predictions for *E. saccharina*. Model predictions were based on a A & C) larval and B & D) pupal over-wintering stage and projected the time steps of life stage transition or generation turn-over over time. "L" on the y axis represents larval instar stage (L_1 = first instar larva).....104

Figure 5.2 The impact of local climate and over-wintering stage on the number of stress hours for the specific life stages during the season. Model outcomes were based on A – B) larval and C – D) pupal over-wintering stages at a warm site and a cold site in South Africa. Horizontal bars denote the life stage relevant for stress hours observed. 108

Figure 5.3 The impact of local climate and over-wintering stage on A & B) adult moth fitness and C & D) relative adult abundance. Model predictions were based on a A & C) larval and B & D) pupal over-wintering stage.....111

Figure 5.4 A) Biophysical thermal stress index (hours), timing of the adult life stage and B) larval instars over time (tallied for a larval over-wintering life stage) are compared to average scout records (expressed as a % larvae scouted) obtained from 29 sugarcane fields (ages 7 – 31 months, 16 different cultivars) in the Eston area (cold site: 29°52′00″S, 30°31′00″E, 785 m.a.s.l). 112

List of tables

Table 2.1. Summary results for temperatures recorded during mid-winter (June) and mid-summer (January) at different locations in adjacent *C. papyrus* and sugarcane planted at Mount Edgecombe, Durban, South Africa. The absolute minimum (Min), absolute maximum (Max), and mean (± SD) temperatures are given in °C per location (n = 962 per locus, per plant) 25

Table 3.1. Geographic sampling locations for *E. saccharina* larvae. Average estimates of the weekly minimum air temperature (Min.), daily air temperature (Mean) and weekly maximum air temperature (Max.) were obtained from the SASRI weatherweb and downloaded on a weekly time resolution for 2012 from weather stations situated close to sampling locations (typically < 18 km). Lat=latitude, Long=longitude. 42

Table 3.2. Summary results from a generalized linear model (with gaussian distribution of errors and an identity link function) of chill-coma onset temperature (°C) among *E. saccharina* populations. Two-day-old adults were measured and compared to the laboratory colony (set as the reference level, or intercept). Model estimates, standard errors (SE), t-values and corresponding *P*–values are given. The residual deviance is 111.76 on 403 degrees freedom and the dispersion parameter is taken to be 0.2773. 47

Table 3.3. Summary results from a generalized linear model (Gaussian distribution of errors and identity link function) of chill-coma induction temperature of *E. saccharina* (CT_{min} in °C) in response to short term (24 hours at 22.0, 27.0 or 32.0 °C) temperature exposure (Acclimation temperature) in combination with adult moth age (two and three day old moths), explored in the laboratory population. Amongst-population variation in short term temperature exposure responses were compared between the warm populations (grouped Malelane and Umfolozi), cold population

(Midlands south) and laboratory stock population. Interaction effects are presented with × and the degrees of freedom (*d.f.*), chi–square (χ^2) statistic, corresponding p–value (*P*) are shown.....49

Table 4.1. Summary of the generalized linear model results determining the effect of developmental acclimation temperature on estimates of body mass (and sex) in adult *Eldana saccharina* moths, adult moth water loss rate (WLR, mg/h), time to death (h) and critical body water content ($BWC_{INITIAL}$, mg). The degrees of freedom (d.f.), chi-square (χ^2) statistic and *P*-values of the model parameters are shown. Units are shown in parentheses after the trait or variable of interest. Significant effects are highlighted in bold. 77

Table 5.1. Thermal constant (K) and lower developmental threshold (LDT) estimates for the different stages, obtained from work done by Way (1995) and estimates of the theoretical optimal temperature (T_{opt}) for development of each life stage (following Briere et al., 1999). 99

Table 5.2. Summary results from a generalized linear model results testing the effects of geographic location (Site) and over-wintering life stage (Stage) on the number of generations, cold- and heat-stress hours, relative adult abundance, fecundity (Poisson distribution of errors and log link function) and relative fitness (Guassian distribution of errors and identity link function). Interaction effects are presented with × and the degrees of freedom (d.f.), chi–square (χ2) statistic, corresponding *P*–value are shown. Significant effects are shown in bold.....105

Chapter 1

General introduction to the physiology of *Eldana saccharina*

(Lepidoptera: Pyralidae)

Temperature, and the tolerance or susceptibility toward it, are central to insect development, performance, distribution, abundance and mortality in the field. Therefore, understanding variation in thermal tolerance of pest insects is critical for population modelling as it is coupled with geographic distribution patterns (Kellermann et al., 2009; Calosi et al., 2010), pest management and in post-harvest control programs (Hallman & Denlinger, 1998; Bale, 2010). The physiological tolerances of populations are often positively correlated with local climate (Kellermann et al., 2012) while compensation for potentially stressful temperatures is evident in continuous physiology and behaviour adjustments to survive and optimize individual fitness in the environment (Terblanche, 2013). At the population scale, thermal adaptation can match geographic distribution and is often associated with geographic environmental gradients. Evidence for the evolution of low temperature performance at the species level (i.e. among populations) is however sparse, particularly in non-model organisms (but see e.g. Kingsolver, 1983; Ayres & Scriber, 1994; Klok & Chown, 2003; Kingsolver et al., 2009).

Depending on the temperature sensitivity of insects, small changes in temperature can result in large differences in metabolic rate, and presumably also respiratory water loss (Addo-Bediako et al., 2002; Dillon et al., 2010; Kearney, 2012). Acclimation of water balance physiology might thus enhance fitness, but it might also come at a cost or lead to sub-optimal trait responses (see discussions in Hoffmann, 1995; Huey & Berrigan, 1996; Deere & Chown, 2006; Terblanche & Kleynhans, 2009).

Eldana saccharina Walker 1865 (Lepidoptera: Pyralidae) is an indigenous graminaceous stem borer of economic importance in many African countries due to larval induced crop losses (Bosque-Pérez, 1995; Polaszek & Khan, 1998; Mazodze & Conlong, 2003). Although much research has focused on control of this borer, it remains a significant agricultural pest in commercially grown sugarcane

(*Saccharum* spp. hybrids) (Poaceae) (Keeping, 2006; Webster et al., 2006; Conlong & Rutherford, 2009; Webster et al., 2009). In Africa, *E. saccharina* occurs naturally in *Cyperus papyrus* L. (Cyperaceae) and a range of other native sedges and grasses (Atkinson, 1980; Polaszek & Khan, 1998; Conlong, 2001). The natural geographic distribution of *E. saccharina* in Africa (Assefa et al., 2006) stretches across a matrix of climates. In South Africa, temperature isotherms were regarded as important distribution modelling tools and the potential distribution of *E. saccharina* was initially thought to be restricted by the 16 °C isotherm across the sugarcane belt of South Africa (Atkinson, 1980). This has been proven to not be so, and *E. saccharina* distribution now stretches far beyond these limits (Assefa et al., 2006, Kleynhans et al., 2014).

This relationship of temperature on the distribution, survival and physiology of *E. saccharina* is further investigated in this thesis. In the first research Chapter, the thermal biology of *E. saccharina* from two of its more common host plants' microclimates, sugarcane or in *C. papyrus,* were studied and how these microclimates varied between seasons (summer and winter). I also aimed to establish baseline knowledge of the thermal limits for activity and survival of *E. saccharina* moths and pupae reared from larvae collected from these two host plants. More specifically, I determined whether thermal limits, including chill-coma induction temperature or critical thermal minima (CT_{min}), high temperatures at which activity ceases or critical thermal maxima (CT_{max}), lower lethal temperature (LLT), and freezing temperature of *E. saccharina* varied between populations living on these different host plants, whilst keeping age, recent diet, and rearing temperature constant in the laboratory. These trait responses have not been reported for field-collected *E. saccharina* from different host plants to date.

In the second research Chapter, among-population variation in CT_{min} in *E. saccharina* were studied, explicitly accounting for a range of potential confounding effects that might bias the

outcome of a comparison amongst populations for CT_{min} variation, including examining and discounting (or controlling) for age, sex, developmental thermal history and short-term thermal acclimation. Specifically aims include determining whether 1) CT_{min} differs between geographic populations and is correlated with local climates, 2) there is evidence of fitness costs of cold tolerance on development time and plastic responses in naturally varying populations and 3) the CT_{min} response is associated with genotypic variation through genetic crosses and polygene calculation.

In the third research Chapter, the two major alternative hypotheses for impacts of rearing temperature on water balance-related traits (e.g. hydration and water economy) on the adult life stage of *E. saccharina* were assessed by exposing immature stages of this species to different rearing temperatures, both above and below optimum conditions, and then measuring the resultant adult physiological performance (water loss rates, time to death) and water-balance related traits (body size, water content). I also sought to assess what traits varied in response to immature stage rearing temperature, and what the net outcome thereof might be for survival of desiccating conditions as adults.

In the fourth and final research Chapter, a mechanism-driven model that consults information on the i) model geographical site(s): and ii) organism life-stage related temperature sensitivity in a commercial sugarcane agriculture system is applied. Based on biophysical principles, the model simulates the microclimatic conditions experienced during each stage of the life history. Biological characteristics of the life stage are coupled to the conditions experienced and the stress indices are calculated. Based on the duration and sensitivity to the stress factors, generation turnover, relative fitness and fecundity are projected. In brief, the aims and objectives of this work allude to first, determining whether critical thermal limits, low lethal temperatures and freezing temperatures are

affected by host-plant. Second, the effects of geographic variation in population abundance on CT_{min} is studied with the objective to determine whether different populations from different climatic histories showed differences in temperature physiology. Third, the ability for *E. saccharina* to adapt to environmental change within a short time were studied, and finally, a mechanism-driven model that incorporates climate data with life stage dependent physiology and predict *E. saccharina* life stage- and generation responses were applied with the main aim to establish whether geographic climate variation and the life stage that was over-wintering significantly affected population outcomes in the field.

1.1 References

Addo-Bediako, A., Chown, S. L., & Gaston, K. J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. Functional Ecology 16: 332–338.

Assefa, Y., Mitchell, A. & Conlong, D. E. (2006) Phylogeography of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Annales de la Société Entomologique de France 42: 331–337.

Atkinson, P. R. (1980) On the biology, distribution and natural host plants of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Journal of the Entomological Society of Southern Africa 43: 171–194.

Ayres, M. P. & Scriber, J. M. (1994) Local adaptation to regional climates in *Papilio canadenis* (Lepidoptera: Papilionidae). Ecological Monographs 64: 465–482.

Bale, J. S. (2010) Implications of cold tolerance for pest management. Low Temperature Biology of Insects (Denlinger, D. L. & Lee, R. E. eds.), pp. 342–373. Cambridge University Press, Cambridge, UK.

Bosque-Pérez, N. A. (1995) Major insect pests of maize in Africa: biology and control. IITA Research Guide 30. Training Program, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 30 p. Second edition.

Calosi, P., Bilton, D. T., Spicer, J. I., Votier, S. C. & Atfield, A. (2010) What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). Journal of Animal Ecology 79: 194–204.

Conlong, D. E. (2001) Biological control of indigenous African stemborers: What do we know. Insect Science and its Application 21: 267–274.

Conlong, D. E. & Rutherford, S. R. (2009) Conventional and New Biological and Habitat Interventions for Integrated Pest Management Systems: Review and Case Studies using *Eldana saccharina* Walker (Lepidoptera: Pyralidae). In: Integrated Pest Management: Innovation-Development Process (Peshin, R. & Dhawan, A. K. eds.), Vol. 1, Chapter 10, pp. 241–261. Springer, Dordrecht, The Netherlands. ISBN 978-1-4020-8991-6 (Print); 978-1-4020-8992-3 (online). DOI 10.1007/978-1-4020-8992-3_10.

Deere, J. A. & Chown, S. L. (2006) Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. The American Naturalist 168: 630–644.

Dillon, M. E., Wang, G. & Huey, R. B. (2010). Global metabolic impacts of recent climate warming. Nature 467: 704–707.

Hallman, G. J. & Denlinger, D. L. (1998) Temperature Sensitivity in Insects and Application in Integrated Pest Management. Westview Press, Boulder, CO, USA.

Hoffmann, A. A. (1995) Acclimation: increasing survival at a cost. Trends in Ecology and Evolution 10: 1–2.

Huey, R. B. & Berrigan, D. (1996) Testing evolutionary hypotheses of acclimation. In: Animals and Temperature. Phenotypic and Evolutionary Adaptation (Johnston, I. A. & Bennett, A. F. eds.), pp. 205–237. Cambridge University Press, Cambridge.

Kearney, M. (2012) Metabolic theory, life history and the distribution of a terrestrial ectotherm. Functional Ecology 26: 167–179.

Keeping, M. G. (2006) Screening of South African sugarcane cultivars for resistance to stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae). African Entomology 14: 277–288.

Kellermann, V. M., Overgaard, J., Hoffmann, A. A., Fløjgaard, C., Svenning, J-C. & Loeschcke, V. (2012) Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. Proceedings of the National Academy of Sciences of the USA 109: 16228–16233.

Kellermann, V. M., van Heerwaarden, B., Sgrò, C. M. & Hoffmann, A. A. (2009) Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. Science 325: 1244–1246.

Kingsolver, J. G. (1983) Thermoregulation and flight in Colias butterflies – elevational patterns and mechanistic limitations. Ecology 64: 53–545.

Kingsolver, J. G., Ragland, G. J. & Diamond, S. E. (2009) Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. Evolution 63: 537–541.

Kleynhans, E., Mitchell, K. A., Conlong, D. E. & Terblanche, J. S. (2014) Evolved variation in cold tolerance among populations of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa. Journal of Evolutionary Biology 27: 1149–1159.

Klok, C. J. & Chown, S. L. (2003) Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. Biological Journal of the Linnean Society 78: 401–414.

Mazodze, R. & Conlong, D. E. (2003) *Eldana saccharina* (Lepidoptera: Pyralidae) in sugarcane (*Saccharum* hybrids), sedge (*Cyperus digitatus*) and bulrush (*Typha latifolia*) in south-eastern Zimbabwe. Proceedings of the South African Sugar Technologists' Association 77: 266–274.

Polaszek, A. & Khan, Z. R. (1998) Host plants. In: African Cereal Stem Borers: Economic Importance, Taxonomy, Natural Enemies and Control (Polaszek, A. ed.), pp. 2–10. CABI, Wallingford, UK.

Terblanche, J. S. & Kleynhans, E. (2009) Phenotypic plasticity of desiccation resistance in *Glossina* puparia: are there ecotype constraints on acclimation responses? Journal of Evolutionary Biology 22: 1636–1648.

Terblanche, J. S. (2013) Thermal relations. In: The Insects: Structure and Function (Simpson, S. J. & Douglas, A. E. eds.), 5th edition by Chapman, R. F., pp. 588–621. Cambridge University Press, Cambridge, UK.

Webster, T. M., Maher, G. W. & Conlong, D. E. (2006) An integrated pest management system for *Eldana saccharina* in the midlands north region of KwaZulu-Natal. Proceedings of the South African Sugar Technologists' Association 79: 347–358.

Webster, T. M., Brenchley, P. G. & Conlong, D. E. (2009) Progress of the area-wide integrated pest management plan for *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in the midlands north region of KwaZulu-Natal. Proceedings of the South African Sugar Technologists' Association 82: 471–485.

Chapter 2

Host plant-related variation in thermal tolerance of *Eldana saccharina* (Lepidoptera: Pyralidae)

Published as Kleynhans, E., Conlong, D. E. & Terblanche, J. S. (2013) Host plant-related variation in thermal tolerance of *Eldana saccharina* (Lepidoptera: Pyralidae). *Entomologia Experimentalis et Applicata* 150: 113-122.

2.1 Introduction

Understanding variation in thermal tolerance of pest insects is critical for pest management. Knowledge of temperature tolerance can be used to determine the developmental limits or mortality thresholds for modelling population dynamics and in post-harvest control programs (Hallman & Denlinger, 1998; Bale, 2010). Growth rates and phenology (i.e., timing of seasonal activities) can be affected by the microclimate temperature experienced in the field (Davidowitz et al., 2004). At longer time-scales, temperatures may directly influence daily survival (Denlinger & Lee, 2010) and population persistence and performance in an inhabited environment or upon introduction to new environments (Régnière et al., 2012).

Strategies of insects for dealing with temperature variation include a wide range of behavioural and physiological compensatory mechanisms (Terblanche, 2013). At high temperatures, for example, insects may avoid overheating through shade-seeking or avoidance behaviour, or increased evaporative cooling. Alternatively, they may rapidly develop biochemical protection, e.g., heat shock proteins, which maintain cell function during or after experiencing potentially damaging conditions (Denlinger & Lee, 2010; Terblanche, 2013). As insects are cooled below their optimum development temperatures, a range of responses are typically recorded, including the cessation of activity and feeding, reduction in neuromuscular responsiveness, chill coma and, potentially, mortality. However, a wide range of temperatures can be withstood between the chill coma induction temperature and the lethal temperature, depending on the low temperature strategy of the species (Chown & Nicolson, 2004). At low temperatures, insects generally employ one of two cold tolerance strategies: freeze tolerance, when extra-cellular ice formation in the body can be tolerated, or freeze susceptibility, in which the lethal effects of freezing can be avoided by lowering the

temperature where body water freezes spontaneously (Sinclair, 1999; Denlinger & Lee, 2010). The temperature at which body water freezes is called the freezing temperature (or supercooling point, SCP) and some insects show marked variation in their freezing or lethal temperatures either through dietary factors (Shreve et al., 2007), presence or absence of food material in the gut, or other physiological adjustments (e.g., thermal acclimatization, body water regulation) (Boardman et al., 2012).

It is increasingly apparent that physiological tolerances of heat and cold may be coupled with an insect's geographic distribution (Kellermann et al., 2009; Calosi et al., 2010), often showing significant positive correlations with local climate (Kellermann et al., 2012). Moreover, biological interactions, such as the presence of obligate symbionts and natural predators associated with host plants, may affect the survival of insect pests (Ferrari et al., 2004; Karley et al., 2004; Dunbar et al., 2007). Alternatively, dietary or nutritional factors may influence thermal limits of insects directly (Shreve et al., 2007; Koštál et al., 2012). In some cases, availability of a suitable host plant may be a significant factor determining population abundance (Pelini et al., 2009), whereas in others climate is thought to be the key determinant of population responses to climate variability (Buckley & Kingsolver, 2012). These interactions between insects and host plants, as well as the variation in microclimates caused by host plants (Pincebourde & Woods, 2012), are therefore important for understanding factors influencing a pest insect's geographic range and potential climate change responses.

Despite the importance of temperature outcomes on *E. saccharina*, the thermal responses of *E. saccharina* from the wild have not been well examined. Here I explored a range of questions on the nature of stalk borer thermal biology which have several potential implications for pest management programs of the species. I aimed to establish whether there is a significant difference

between the microclimate that *E. saccharina* experiences when in sugarcane (usually occupying the lower one-third section of the stalk), or in *C. papyrus* (usually occupying the umbel, which is at the top of the plant) (Conlong, 1990), and how these microclimates vary between seasons (summer and winter). Eggs, larvae, and pupae of *E. saccharina* reside in these microsites throughout the year. The larval life-stage lasts for ca. 30 days and the environmental characteristics experienced during this time may play a significant role during the later life stages (e.g. Terblanche & Chown, 2006), via mating and reproduction (in adults), or via survival because they are immobile, and probably over-wintering (in pupae). I also specifically aimed to establish baseline knowledge of the thermal limits for activity and survival of *E. saccharina* moths and pupae reared from larvae collected from two host plants. Specifically, I aimed to determine whether thermal limits, including chill-coma induction temperature (= critical thermal minimum), high temperatures at which activity ceases (= critical thermal maxima), lower lethal temperature, and freezing temperature of *E. saccharina* vary when populations have been living on different host plants, whilst keeping age, recent diet, and rearing temperature constant in the laboratory. These trait responses have not been reported for field-collected *E. saccharina* from different host plants to date.

2.2 Materials and methods

2.2.1 Study organisms and rearing conditions

Wild larvae were collected close to Eston in the Southern Midlands of KwaZulu Natal, South Africa (29.847°S, 30.523°E; 704 m above sea level) from a natural host plant (*C. papyrus*) and adjacent cultivated sugarcane for comparisons of thermal tolerance traits of several *E. saccharina* life-stages. Wild, field-collected larvae (n = 60-80 larvae per host, including instars 1-5) were placed individually into 30-ml vials containing a species-specific artificial diet medium (see Table 6 in

Gillespie, 1993) and kept in a cool, dry container while transported back to the rearing facility in Durban (South African Sugarcane Research Institute, Mount Edgecombe). Upon arrival at the rearing facility, the larvae in their collection vials were kept in a quarantine room maintained at 24 ± 2 °C and 65 ± 5% relative humidity. Upon emergence, moths were collected and transferred to a rearing room kept at a constant 26 ± 2 °C, 75 ± 5% relative humidity, and L12:D12 h photoperiod. The photoperiod matched the average natural light cycles during June – November (duration of this study) in the areas of collection. Moths were placed into sterile (ca. 30 × 30 × 12 cm) clear perspex boxes lined with black plastic to prevent oviposition in the box corners, and provided with white tissue paper as oviposition substrate. A 250-ml container with fresh water and four dental wicks through holes in the lid of the container provided the moths with water ad libitum. Mating, completion of the gestation period, and egg laying took place within the boxes. Differences in thermal trait responses between generations were not investigated. However, care was taken to ensure that the first set of replicates (n = 10 individuals per gender and host plant) of each experiment was not significantly different from the second and third replicates for each physiological measurement, thus achieving a total sample size of 30 male and 30 female moths per host plant. To avoid maternal or inter-generational acclimation responses or laboratory adaptation which may have confounded measurement of thermal trait responses, I completed measurements within the first 90 days (ca. two generations, typically between days 50 and 90 in the laboratory) of common-garden rearing. I therefore consider the results a reasonable reflection of wild, but standardized population responses.

2.2.2 Physiological responses

The critical thermal minima (CT_{min}) and maxima (CT_{max}) were measured on individual moths at 2 days after emergence for each gender, because age may have a marked impact on thermal tolerance

of insects (Bowler & Terblanche, 2008). Moths that were assayed for chill-coma induction temperature were reared under common environmental conditions (i.e., common garden) from larvae collected from either *C. papyrus* (n = 50) or sugarcane (n = 60). To control for nutritional effects or possible short-term thermal adjustments influencing thermal limits, moths assayed were reared to at least the F_2 generation on artificial diet and under controlled conditions (26 ± 2 °C, 75 ± 5% relative humidity) as described above. However, CT_{max} does not generally change in such a rapid plastic manner, or to such a great extent as chill-coma or low temperature activity traits (e.g. Terblanche & Chown, 2006). I therefore measured the maximum thresholds of wild moths upon eclosion from pupae (after 24 h with water *ad libitum*) reared from larvae collected from *C. papyrus* (n = 44) or sugarcane (n = 26). Pupation continued in the quarantine room (see above).

Critical thermal limits (CTL) of moths were determined by inserting individuals into a double-jacketed air filled insulated chamber coupled to a programmable water bath, which allowed for controlled heating or cooling, in order to measure CTL. The chambers formed part of an 'organ-pipe' unit, connected to a fluid circulated controllable refrigeration bath (GP150-R2; Grant Instruments, Cambridge, UK). The fluid circulates around the 11 chambers (10 insects, one control chamber for monitoring temperature) and returns to the bath, thereby controlling the temperature experienced inside each chamber. A fine, type-T thermocouple (5SC-TT-T-36-36; Omega Engineering, Stamford, CT, USA) was inserted into the control chamber to ensure the desired temperatures were achieved and for noting temperature upon reaching the CTL. The thermocouple was connected, via a TC-08 Picologger multi-plexer, Pico Technology, Cambridgeshire, UK, to a computer acting as a data recorder.

The CT_{min} (lower CTL or chill coma temperature) were defined as the point where reduced motor function occurred (i.e., spastic muscle movements) and the moths were unable to cling to a

paintbrush. The CT_{max} (upper CTL) represented the temperature at which muscle function was lost, typically accompanied by a loss of movement or neuromuscular control. After an equilibration time of 10 min at either 20 or 30 °C, temperature was 'ramped' down for CT_{min}, or up for CT_{max} at a constant ramping rate of 0.1 °C per min, respectively. Moths were prodded gently with a soft paintbrush at regular intervals to identify the endpoints of the assay. Individual moths were continuously subjected to the assays (i.e., never removed from the chambers) during the trials until the CTL were reached and no moths were ever re-used for other trials.

All statistical analyses were undertaken in R, v. 2.15.1 (R Development Core Team, 2010). CT_{min} data did not meet the assumption of normality (Shapiro-Wilk W = 0.9552, P<0.001), whereas the CT_{max} data were normally distributed (Shapiro-Wilk W = 0.9753, P = 0.18). Comparisons of CT_{min} between host plants were therefore undertaken using a Wilcoxon rank-sum statistic after the main and interaction effects of sex and host plant were analyzed using a generalized linear model (GLZ) with a normal probability distribution of errors and an identity link function. CT_{max} data were compared between host plants using a linear model (to test for the main or interaction effect of sex) and Student's independent t-test (Welch two sample t-test). The data met the assumption of homoscedasticity (equal variances: verified using Levene's F-tests) despite some differences in sample size. A lack of overlap of box-plot notches was used to identify significant differences in medians on a 95% confidence level (following Crawley, 2007).

The LLT of moths was assessed using a standard 'plunge' protocol (e.g. Sinclair et al., 2006). Moths used were collected during the larval stage and reared under controlled, constant conditions (27 ± 1 °C, 76 ± 5% relative humidity) for at least two generations (n = 300 from *C. papyrus*, n = 310 from sugarcane). Empty 35-ml vials containing 2-day-old moths were placed inside a plastic bag placed inside a circulating controllable refrigeration bath (GP150-R2). Each temperature

treatment lasted 2 h. After the treatment, moths were transferred to a ventilated container with water-saturated cotton wool providing water ad libitum. Survival, defined as a coordinated response to gentle prodding, was scored after 24 h at a constant, optimal rearing temperature (27 ± 1 °C, 76 ± 5% relative humidity). The range of conditions tested always encompassed the full range of moth survival from 0-100%, covering a temperature range of -10 to +10 °C. A fine type-T thermocouple (5SC-TT-T-36-36) was placed inside the 35-ml vial, and the vial inside the plastic bag to ensure that the desired treatment conditions were reached. The thermocouple was connected via a multi-plexer (Pico TC-08) to a computer acting as a data recorder using PicoLog software so that the temperature trace could be seen on the computer. Survival was scored as a percentage survival out of at least 10 individuals and each container was replicated at least three times per treatment temperature and sex. The LLT of 90 and 50% of the population (LT_{90} and LT_{50}, respectively) were identified from the fitted logistic regression in R [using the dose.p function (Venables & Ripley, 2002) in the MASS library].

For statistical analyses, I tested the main and interaction effects of sex and temperature per host plant. I found that the main and interaction effects of sex were not significant, and therefore did not include the sex effect into the full model. I tested the main and interaction effect of host plant and temperature using a GLZ. The non-linear effect of temperature on moth survival was determined using a logistic regression with a binomial distribution of errors and probit link function with vial as the unit of measurement. To determine whether freezing was coupled with death in *E. saccharina* moths, I carried out a supercooling point (SCP) assay combined with a mortality essay. The SCP was identified by detection of the released latent heat of crystallization (Sømme, 1999). To measure the SCP individually, an individual specimen was placed firmly against a fine type-T thermocouple with cotton wool inside a 1.5-ml plastic tube. This experimental setup allowed association between each specimen and its temperature trace. The thermocouple was connected via a TC-08 Picologger

multi-plexer to a computer acting as a data recorder. Temperature of the bath was ramped down at a constant cooling rate of 0.1 °C per min after an equilibration time of 10 min at 5 °C. The cooling period was recorded at an interval of 30 s using PicoLog software and SCP was recorded as the immediate temperature prior to the spike in temperature, i.e., exotherm, observed on the thermal trace (Lee, 1991). I only included moths reared from larvae that were collected from sugarcane in this trial. Moths were removed from the water bath prior to freezing (at ca. -10 °C; n = 30) or upon SCP detection (n = 27). Survival was scored in the two groups (i.e., the group which froze and went below SCP and the group which had not frozen) after 24 h with water ad libitum.

The pupae used to measure the minimum freezing temperature of *E. saccharina* were taken from field-collected larvae that had pupated in their field collection vials in the quarantine room. The SCP data did not meet the assumption of normality (Shapiro-Wilk W = 0.778, $P < 0.001$). Comparisons of SCPs between host plants were undertaken using a Wilcoxon rank-sum statistic, correcting for a normal approximation for the *P*-value.

2.2.3 Microclimate data

Calibrated thermochron iButton data loggers (8-bit Model DS1921; iButton, Dallas, TX, USA; 0.5 °C accuracy) were used to record microclimate temperatures at 30-min sampling frequencies at three locations within sugarcane (age 7 - 9 months) and adjacent *C. papyrus* (2 - 3 m high) from which *E. saccharina* larvae were collected. Sugarcane microclimate temperature was measured at the stalk roots inside the soil, 30 cm above the ground behind the dead leaf sheath in a second stalk, and 10 cm above the ground inside the stalk centre of a third stalk. The three sugarcane stalks were from the same ratoon, within a 15-cm radius from each other. Measurements of *C. papyrus* microclimates were recorded similarly at three locations in three separate culms and umbels: above,

in, and directly under the *C. papyrus* umbel, i.e., a culm and umbel were used per location to result in three replicates per plant type per season. Measurements were taken during a mid-winter month (June 2012) and mid-summer month (January 2013) for 21 consecutive days at 30-min intervals. The iButtons were never placed in direct sunlight; iButtons above *C. papyrus* umbels were well insulated at the base of the ray. Owing to the non-parametric nature of the data, and the fact that temperature data are temporally autocorrelated, the microclimate data were analyzed using a Wilcoxon matched pairs test.

2.3 Results

2.3.1 Physiological responses

There were significant differences in median CT_{min} (non-normal frequency distribution) and mean CT_{max} (normal frequency distribution) values of individuals collected from the two host plants, respectively (W = 2 916, $P < 0.0001$; Figure 2.1A; t = 10.36, d.f. = 66.96, $P < 0.0001$; Figure 2.1B). Estimates of CT_{min} for male moths are similar to that of female moths (GLZ: $\chi^2 = 0.06$, d.f. = 1, $P = 0.81$), and no interaction effect of gender was found with host plants (GLZ: $\chi^2 = 0.643$, d.f. = 1, $P = 0.42$). Estimates of CT_{max} were independent of sex (LM: $F_{1,66} = 2.44$, $P = 0.12$), and no interaction effect of gender was found with host plants (LM: $F_{1,66} = 3.07$, $P = 0.084$). When tested separately, the CT_{max} data from the *C. papyrus* population did not meet the assumption of normality (Shapiro-Wilk W = 0.9083, $P < 0.01$), however the data from the sugarcane population were normally distributed (Shapiro-Wilk W = 0.9576, $P = 0.35$). The mean CT_{max} measured in the *C. papyrus* population was significantly higher than the mean CT_{max} recorded in the sugarcane population (mean = 44.9 °C in *C. papyrus* vs. 44.6 °C in sugarcane; Welch two sample t-test t = 10.4, d.f. = 67, $P < 0.001$) (Figure 2.1B).

Lower lethal temperature assays showed that there were no significant differences between male and female moths and no significant interaction between sex and temperature effects in the *C. papyrus* (GLZ: χ^2 = 1.078, d.f. = 4, P = 0.90) or sugarcane (χ^2 = 0.122, d.f. = 4, P > 0.99) populations. The main effect of temperature explained the variation in the mortality data best (χ^2 = 240.9, d.f. = 4, P < 0.0001), whereas the main effect of host plant was not significant (χ^2 = 0, d.f. = 1, P > 0.99) and the interaction effect between temperature and host plant was not significant (χ^2 = 3.0, d.f. = 4, P = 0.56). LT_{90} and LT_{50} from sugarcane populations determined from logistic regression were (means ± SE) -10.0 ± 0.9 and -3.2 ± 0.5 °C in comparison to -8.9 ± 0.8 and -3.9 ± 0.4 °C from *C. papyrus* (Figure 2.1C).

Freezing was lethal in *E. saccharina* moths (mean freezing temperature: -15.3 ± 0.2 °C) and survival prior to freezing was significantly higher than upon freezing (Figure 2.2). In pupae, no significant differences in median SCP values between host plants were found (W = 140, P = 0.25; Figure 2.2).

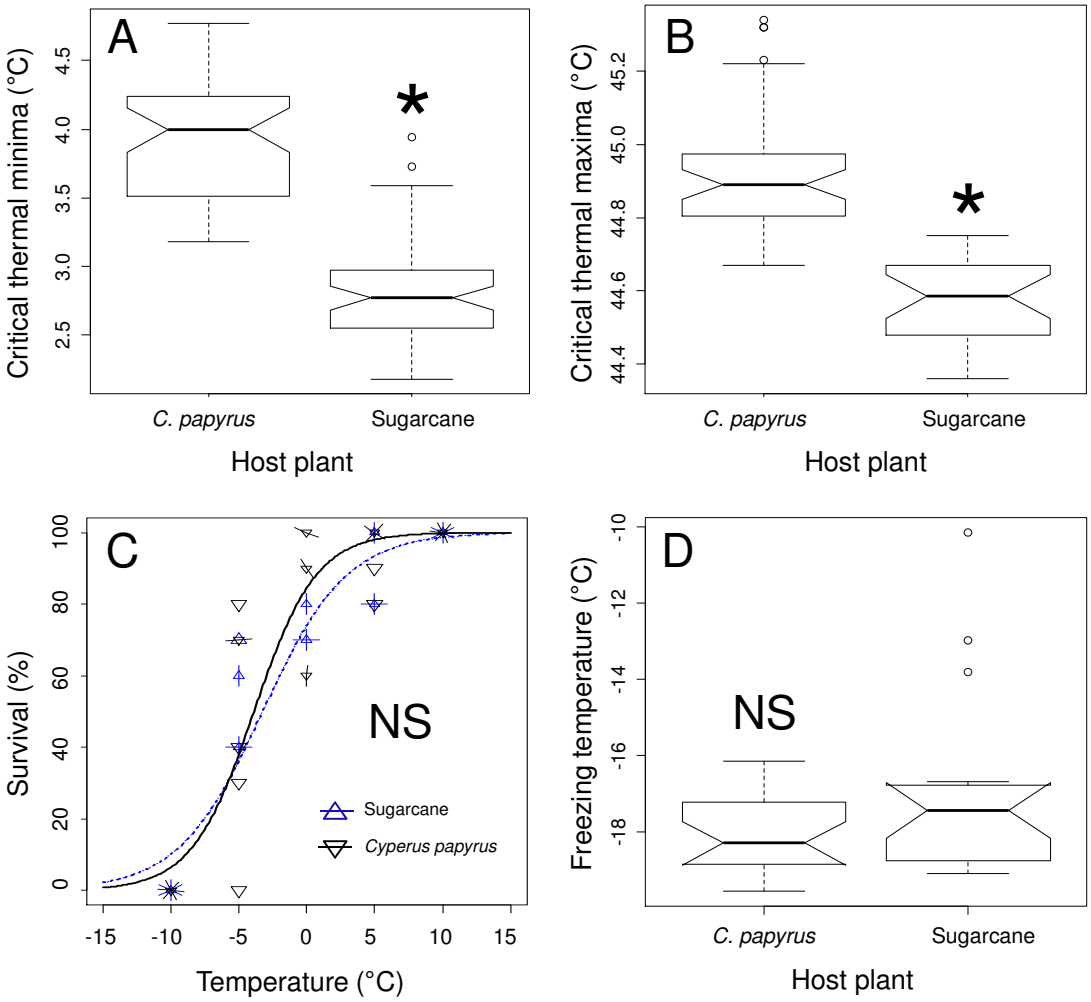

Figure 2.1. Host-plant induced variation in (A) *E. saccharina* moth critical thermal minima, (B) moth critical thermal maxima, (C) logistic curve of the lower lethal temperatures of moths originally from *C. papyrus* (black upside down triangles, solid line) or sugarcane (blue upright triangles, stippled line), and (D) puparial freezing (supercooling point) temperatures. Box-plots summarize the median (horizontal line), 25th and 75th percentiles [i.e., location of the middle 50% of the data (bottom and top of the box)] and the minimum – maximum data range [when there are no outliers, else, 1.5 times the interquartile ranges (whiskers)]. Outliers, identified as data points more than 1.5 times the interquartile range, are plotted individually. Notches, drawn as a 'waist' in a triangular shape from both sides of the median give an indication of a significant difference

between two medians on a 95% confidence interval (CI) when no overlap occurs between two plots' notches (i.e. triangle height indicates 95% CIs). The asterisks in panels A and B indicate significant differences between host plants ('ns' indicates no significant difference). In panel C, multiple points are plotted as 'sunflowers' with multiple 'leaves' indicating overplotting (R, graphics package).

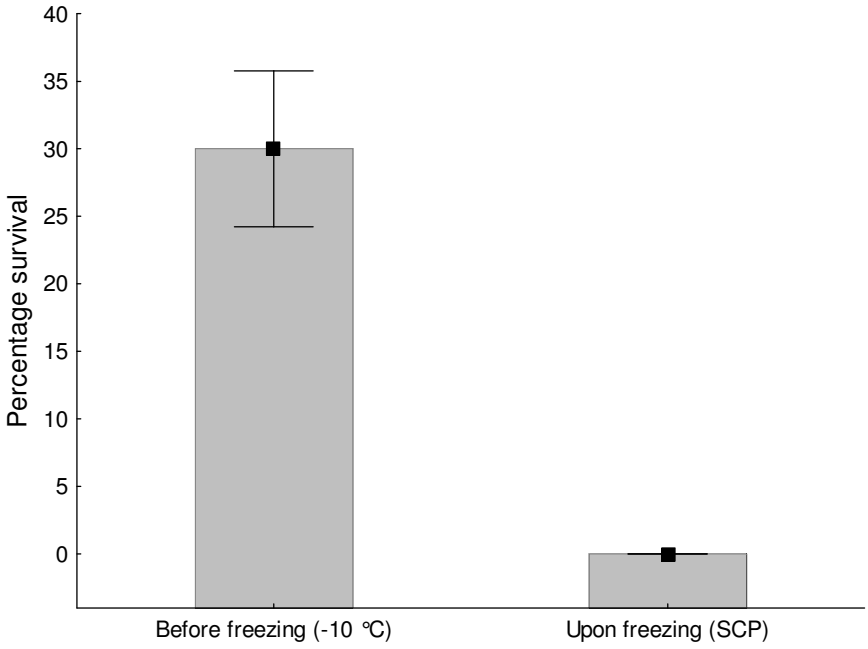

Figure 2.2 To determine whether freezing was coupled with death in *E. saccharina* moths I carried out a supercooling point (SCP) mortality essay. Moths were removed at -10 °C (n = 30; removed before freezing) from a standard 'ramping' treatment (0.1 °C per min from 5.0 °C) and upon observation of the exotherm (n = 27; removed upon freezing). Mortality was scored after 24 h at rearing conditions with water provided *ad libitum*. No moths survived after removal upon freezing.

2.3.2 Microclimate data

There were no significant differences in temperature in *C. papyrus* microsites (winter: $\chi^2 = 2.60$, d.f. = 2, $P = 0.27$; summer: $\chi^2 = 5.67$, d.f. = 2, $P = 0.059$; Table 2.1). However, the microsites (locations of microclimate measurement) in sugarcane were significantly cooler (winter: $\chi^2 = 10.53$, d.f. = 2, $P = 0.0052$; summer: $\chi^2 = 19.10$, d.f. = 2, $P < 0.0001$). In *C. papyrus*, the absolute amount of temperature variation (maximum – minimum) was 28.5 and 27.0 °C during the winter and summer months, respectively. In sugarcane, the amount of temperature variation was 26.0 (winter) and 16.0 °C (summer). The daily minimum temperatures were always higher in sugarcane than in *C. papyrus*, and, when comparing the winter medians of the two host plants, the differences were significant (Wilcoxon signed rank test: $P < 0.001$).

Table 2.1. Summary results for temperatures recorded during mid-winter (June) and mid-summer (January) at different locations in adjacent *C. papyrus* and sugarcane planted at Mount Edgecombe, Durban, South Africa. The absolute minimum (Min), absolute maximum (Max), and mean (± SD) temperatures are given in °C per location (n = 962 per locus, per plant)

Season	Plant and locus	Min	Max	Mean
Winter	*C. papyrus* (bottom of umbel)	4.5	30.5	17.0 ± 5.8
	C. papyrus (middle of umbel)	4.5	29.0	16.7 ± 5.3
	C. papyrus (top of umbel)	3.5	32.0	16.8 ± 6.4
	Sugarcane (bottom of stalk at soil level)	8.5	31.0	16.6 ± 3.1
	Sugarcane (30cm above ground in leaf sheaths)	5.0	31.0	16.8 ± 5.1
	Sugarcane (10cm above the ground inside stalk)	5.5	31.0	16.3 ± 4.5
Summer	*C. papyrus* (bottom of umbel)	18.0	38.0	25.5 ± 4.8
	C. papyrus (middle of umbel)	17.0	44.0	26.1 ± 6.3
	C. papyrus (top of umbel)	18.0	38.0	25.3 ± 4.6
	Sugarcane (bottom of stalk at soil level)	20.5	28.0	24.0 ± 1.7
	Sugarcane (30cm above ground in leaf sheaths)	18.0	34.0	24.7 ± 3.6
	Sugarcane (10cm above the ground inside stalk)	18.5	33.0	24.3 ± 2.9

2.4 Conclusion

Insects typically occupy thermal niches that are ideal for short-term performance and long-term population persistence. Low temperatures, characteristic of winter, can influence survival directly (e.g., via chill injury or freezing-related damage) or indirectly by influencing population dynamics (through e.g., suppressed activity or delayed development and reproduction) which together may have marked effects on the prediction of pest species' responses to field temperature variation (Lee, 1991; see e.g., Nyamukondiwa et al., 2013). In order to understand and further investigate possible behavioural regulation, physiological adaptation or long-term changes in key performance traits that may affect *E. saccharina* survival and population responses, the thermal tolerance of *E. saccharina* in the field requires attention. At longer time-scales, upper and lower thermal limits for development have been reported for *E. saccharina* previously (Way, 1994). Here, I present acute measures of heat and cold tolerance and survival which, until now, have been lacking for this species. In addition, I examined the potential influence of host plant on thermal limits to activity and survival in *E. saccharina* – a key issue which has been generally poorly explored in these and other insects to date. Although it is possible that our method of keeping all insects from the two host plants on the same artificial diet might introduce a confounding factor (e.g. the artificial diet may be sub-optimal in some way) thereby affecting the thermal traits examined, it is difficult to overcome this issue and the alternative approach of direct comparisons made immediately upon collection from the field is confounded by a range of other factors, such as a lack of control for recent thermal history and age (reviewed in Bowler & Terblanche, 2008; Terblanche et al., 2011). I therefore consider our results as an important demonstration of the effect of host plant on lower thermal limits to activity (but not the other low temperature traits examined) despite the individuals having been reared on common artificial diet for a substantial period of time. The advantage of the present study design is that it allowed us to eliminate a suite of other potential confounding factors known for

their influence on thermal traits. Although it is clear that methodology has a marked impact on the outcomes of thermal assays (reviewed in Terblanche et al., 2011), the results of these laboratory trials can provide insights into activity and lethal limits under certain field conditions, which in turn can be of direct value in applied pest management programs (e.g. Chidawanyika & Terblanche, 2011; reviewed in Sørensen et al., 2012).

Variation in CT_{min} of *E. saccharina* collected from sugarcane and *C. papyrus* plants persisted after rearing under common laboratory conditions for up to 90 days (1-2 generations) and even following the elimination of the potential confounding effects of variation in age and water availability. Two main possibilities stand central to explaining this variation. First, from an evolutionary perspective, different thermal environments could result in natural selection for physiological changes that result in traits being associated with local microclimates (Kleynhans & Terblanche, 2009; Kellermann et al., 2012). Second, dietary effects and nutritional differences from feeding on different host plants might result in differences in abiotic stress tolerance (via, e.g., the gut content's acting as an ice nucleating agent or nutritional factors; Boardman et al., 2012). The data show significant evidence for microclimatic variation between the host plants. However, the CT_{min} responses between the host plants did not match the direction of microclimatic variation. For example, winter minimum temperatures were on average 2.2 °C lower in *C. papyrus* than in sugarcane, yet moths from *C. papyrus* showed a higher chill-coma induction temperature than specimens collected from sugarcane (median: 4.0 vs. 2.8 °C). This suggests that the host plant can allow a species to break the evolutionary microclimate 'rule', and thus, that other factors, such as plant chemical composition (e.g., stoichiometry; Jensen et al., 2006) or other indirect effects of host plant on the insect (e.g., nutrition or immune response; Rolff & Siva-Jothy, 2003) might affect thermal activity limits in these moths which may ultimately dominate over the influence of thermal background (see also Coggan et al., 2011).

The second main finding of this study was that there was significant variation in CT_{max} between individuals collected from the two host plants after rearing under common conditions. Summer maximum temperatures were on average 8.4 °C warmer in *C. papyrus* than in sugarcane. Specimens from *C. papyrus* showed a small but significantly higher activity threshold (CT_{max}) than specimens collected from sugarcane (median: 44.9 vs. 44.6 °C). Although the direction of trait variation between CT_{max} responses masked the summer month microclimate observations, the magnitude of the trait variation is small (ca. 0.3 °C) in comparison to the CT_{min} variation (ca. 1.2 °C) potentially associated with host plant. The lower degree of trait variation in CT_{max} can be explained by low plasticity and low evolutionary adaptability (i.e., constraints) in CT_{max}, as observed generally across insects and other vertebrate ectotherms (Hoffmann et al., 2013).

The third main finding of this study was the lack of variation in moth LLT and puparial SCP between host plants, possibly suggesting limited variation in freeze-tolerance strategy with host plant, although a fuller examination of all life-stages for their freezing strategy would be useful. Low-temperature mortality assays confirmed that freezing caused death in *E. saccharina* moths. Freezing occurred well below -10.0 °C for moths and pupae, indicating that *E. saccharina* can probably over-winter in areas where frost occurs, which is typical of parts of this species' geographic range in South Africa, and which may allow it to increase its range into areas previously thought too cold for its survival. This suggests the potential thermal trait responses to host plant are trait specific, as might generally be expected if these traits are under different genetic control (e.g., Anderson et al., 2005). Under field conditions, however, *E. saccharina*'s freezing temperature may well be substantially higher owing to their gut contents potentially acting as an ice-nucleating agent (Boardman et al., 2012). Further work is required to examine SCP and LLT in the field to determine whether low-temperature mortality may be critical to overwintering of local populations and whether freeze tolerance strategy varies among life-stages and seasons.

Finally, absolute minimum soil temperatures were higher (ca. 3.2 °C) than minimum temperatures in the leaf sheath or inside the sugarcane stalk during winter suggesting that these plants do not necessarily buffer soil conditions. In summer, however, the absolute maximum soil temperatures were lower (ca. 5.5 °C) than in the leaf sheath or inside the stalk. The ability of *E. saccharina* to exploit less-variable climatic environments, as in sugarcane, might improve survival and population persistence through a reduction in the temperature extremes that are experienced. For example, unseasonal frost and diurnal fluctuation in microclimates could affect the phenology of *E. saccharina* in the KwaZulu-Natal Midlands.

2.5 References

Anderson, A. R., Hoffmann, A. A. & McKechnie, S. W. (2005) Response to selection for rapid chill coma recovery in *Drosophila melanogaster*: physiology and life-history traits. Genetical Research 85: 15–22.

Bale, J. S. (2010) Implications of cold tolerance for pest management. Low Temperature Biology of Insects (Denlinger, D. L. & Lee, R. E. eds.), pp. 342–373. Cambridge University Press, Cambridge, UK.

Boardman. L., Grout, T. & Terblanche, J. S. (2012) False codling moth *Thaumatotibia leucotreta* (Lepidoptera, Tortricidae) larvae are chill-susceptible. Insect Science 19: 315–328.

Bowler, K. & Terblanche, J. S. (2008) Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? Biological Reviews 83: 339–355.

Buckley, L. B. & Kingsolver, J. G. (2012) The demographic impacts of shifts in climate means and extremes on alpine butterflies. Functional Ecology 26: 969–977.

Calosi, P., Bilton, D. T., Spicer, J. I., Votier, S. C. & Atfield, A. (2010) What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). Journal of Animal Ecology 79: 194–204.

Chidawanyika, F. & Terblanche, J. S. (2011) Costs and benefits of thermal acclimation for codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): Implications for pest control and the sterile insect release programme. Evolutionary Applications 4: 534–544.

Chown, S. & Nicolson, S. W. (2004) Insect Physiological Ecology: Mechanisms and Patterns. Oxford University Press, Oxford, UK.

Coggan, N., Clissold, F. J. & Simpson, S. J. (2011) Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes. Proceedings of the Royal Society of London B 278: 2745–2752.

Conlong, D. E. (1990) A study of pest-parasitoid relationships in natural habitats: an aid towards the biological control of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in sugarcane. Proceedings of the South African Sugar Technologists' Association 64: 111–115.

Crawley, M. J. (2007) The R Book. John Wiley and Sons, Chichester, UK.

Davidowitz, G., D'Amico, L. J. & Nijhout, H. F. (2004) The effects of environmental variation on a mechanism that controls insect body size. Evolutionary Ecology Research 6: 49–62.

Denlinger, D. L. & Lee, R. E. (2010) Low Temperature Biology of Insects. Cambridge University Press, Cambridge, UK.

Dunbar, H. E., Wilson, A. C. C., Ferguson, N. R. & Moran, N. A. (2007) Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. PloS Biology 5: 1006–1015.

Ferrari, J., Darby, A. C., Daniell, T. J., Godfray, H. C. J. & Douglas, A. (2004) Linking the bacterial community in pea aphids with host-plant and natural enemy resistance. Ecological Entomology 29: 60–65.

Gillespie, D. (1993) Development of mass-rearing methods for the sugarcane borer *Eldana saccharina* (Lepidoptera: Pyralidae). 2: Diet gelling agents. Proceedings of the South African Sugar Technologists' Association 67: 127–129.

Hallman, G. J. & Denlinger, D. L. (1998) Temperature Sensitivity in Insects and Application in Integrated Pest Management. Westview Press, Boulder, CO, USA.

Hoffmann, A. A., Chown, S. L. & Clusella-Trullas, S. (2013) Upper thermal limits in terrestrial ectotherms: how constrained are they? Functional Ecology 27: 934–949.

Jensen, T. C., Leinaas, H. P. & Hessen, D. O. (2006) Age-dependent shift in response to food element composition in Collembola: Contrasting effects of dietary nitrogen. Oecologia 149: 583–592.

Karley, A. J., Parker, W. E., Pitchford, J. W. & Douglas, A. E. (2004) The mid-season crash in aphid populations: why and how does it occur? Ecological Entomology 29: 383–388.

Kellermann, V. M., Overgaard, J., Hoffmann, A. A., Fløjgaard, C., Svenning, J-C. & Loeschcke, V. (2012) Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. Proceedings of the National Academy of Sciences of the USA 109: 16228–16233.

Kellermann, V. M., van Heerwaarden, B., Sgrò, C. M. & Hoffmann, A. A. (2009) Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. Science 325: 1244–1246.

Kleynhans, E. & Terblanche, J. S. (2009) The evolution of water balance in *Glossina* (Diptera: Glossinidae): correlations with climate. Biology Letters 5: 93–96.

Koštál, V., Šimek, P., Zahradníčková, H., Cimlová, J. & Štětina, T. (2012) Conversion of the chill susceptible fruit fly larva (*Drosophila melanogaster*) to a freeze tolerant organism. Proceedings of the National Academy of Sciences of the USA 109: 3270–3274.

Lee, R. E. Jr (1991) Principles of insect low temperature tolerance. In: Insects at Low Temperature (Lee, R. E. Jr & Denlinger, D. L. eds.), pp. 17–46. Chapman and Hall, New York, NY, USA.

Nyamukondiwa, C., Weldon, C. W., Chown, S. L., le Roux, P. C. & Terblanche, J. S. (2013) Thermal biology, population fluctuations and implications of temperature extremes for the management of two globally significant insect pests. Journal of Insect Physiology 59: 1199–1211.

Pelini, S. L., Dzurisin, J. D. K., Prior, K. M., Williams, C. M., Marsico, T. D. et al. (2009) Translocation experiments with butterflies reveal limits to enhancement of poleward populations

under climate change. Proceedings of the National Academy of Sciences of the USA 106: 11160–11165.

Pincebourde, S. & Woods, A. H. (2012) Climate uncertainty on leaf surfaces: the biophysics of leaf microclimates and their consequences for leaf-dwelling organisms. Functional Ecology 26: 844–853.

R Development Core Team (2010) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Régnière, J., Powell, J., Bentz, B. & Nealis, V. (2012) Effects of temperature on development, survival and reproduction of insects: Experimental design, data analysis and modeling. Journal of Insect Physiology 58: 634–647.

Rolff, J. & Siva-Jothy, M. T. (2003) Invertebrate ecology immunology. Science 301: 472–475.

Shreve, S. M., Yi, S-X. & Lee, R. E. (2007) Increased dietary cholesterol enhances cold tolerance in *Drosophila melanogaster*. Cryo-Letters 28: 33–37.

Sinclair, B. J. (1999) Ice nucleation and freezing in New Zealand alpine and lowland weta, *Hemideina* spp. (orthoptera; Stenopermatidae). Physiological Entomology 24: 56–63.

Sinclair, B. J., Terblanche, J. S., Scott, M. B., Blatch, G. L., Klok, C. J. & Chown, S. L. (2006) Environmental physiology of three species of Collembola at Cape Hallett, North Victoria Land, Antarctica. Journal of Insect Physiology 52: 29–50.

Sømme, L. (1999) The physiology of cold hardiness in terrestrial arthropods. European Journal of Entomology 96: 1–10.

Sørensen, J. G., Addison, M. F. & Terblanche, J. S. (2012) Mass-rearing of insects for pest management: Challenges, synergies and advances from evolutionary physiology. Crop Protection 38: 87–94.

Terblanche, J. S. & Chown, S. L. (2006) The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae). Journal of Experimental Biology 209: 1064–1073.

Terblanche, J. S. (2013) Thermal relations. In: The Insects: Structure and Function (Simpson, S. J. & Douglas, A. E. eds.), 5th edition by Chapman, R. F., pp. 588–621. Cambridge University Press, Cambridge, UK.

Terblanche, J. S., Hoffmann, A. A., Mitchell, K. A., Rako, L., le Roux, P. C. & Chown, S. L. (2011) Ecologically relevant measures of tolerance to potentially lethal temperatures. Journal of Experimental Biology 214: 3713–3725.

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S, 4th edn. Springer, New York, NY, USA.

Way, M. J. (1994) A preliminary assessment of the effects of different constant temperatures on the reproduction of *Eldana saccharina* (Lepidoptera: Pyralidae). Proceedings of the South African Sugar Technologists' Association 68: 16–18.

Chapter 3

Evolved variation in cold tolerance among populations of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa

Published as Kleynhans, E., Mitchell, K. A., Conlong, D. E. & Terblanche, J. S. (2014) Evolved variation in cold tolerance among populations of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa. *Journal of Evolutionary Biology* 27: 1149-1159.

3.1 Introduction

Species geographic distributions are thought to be linked to functional traits through environmental niches. One key aspect of climate potentially limiting a species' distribution is low temperature performance or tolerance (Addo-Bediako et al., 2000; Jenkins & Hoffmann, 2001; Kellermann et al., 2009; Overgaard et al., 2011). Evolved variation in low temperature stress resistance is typically demonstrated as differences between species over large geographic and evolutionary scales (Kimura, 2004; Kellermann et al., 2009; Hoffmann et al., 2013). Natural selection can promote adaptive trait variation (Ghalambor et al., 2007, Huey, 2010, Overgaard et al., 2010; Overgaard et al., 2011) and thermal trait variation is therefore expected to contribute to evolutionary fitness (Huey & Stevenson, 1979; Kingsolver, 2009). In such cases, allele frequencies are expected to change as a result of selection (e.g. Bijlsma & Loeschcke, 2005; David et al., 2005; Overgaard et al., 2010). In effect, thermal adaptation can match geographic distribution and is often associated with geographic environmental gradients. Evidence for the evolution of low temperature performance at the species level (i.e. among populations) is however sparse, particularly in non-model organisms (but see e.g. Ayres & Scriber, 1994; Kingsolver, 1983; Klok & Chown, 2003; Kingsolver et al., 2009). These studies often focus on associations of tolerance and performance with environmental gradients (i.e. altitudinal and latitudinal clines, Hoffmann et al., 2002; Klok & Chown, 2003). Understanding inter-population variation is important for the prediction of potential climate change-related responses across landscapes (Hill et al., 2012; Sinclair et al., 2012) and for understanding the initial steps of speciation (Ghalambor et al., 2007) and potential niche shifts (Hill et al., 2013).

There is much variation among low temperature tolerance traits of insects (see Hoffmann et al., 2013), potentially reflecting systematic methodological or genetic differences (see Discussion in

e.g. Terblanche et al., 2011 for high temperature tolerance; and see Anderson et al., 2005; Rako et al., 2007). Reversible paralysis that occurs at the CT_{min} may be due to the loss of ion homeostasis in the neuromuscular system that causes signal transmission disruption, and therefore represents a measure of the functional lower limits to activity and performance (Anderson & Mutchmor, 1968; Goller & Esch, 1990; reviewed in Hazell & Bale, 2011; MacMillan & Sinclair, 2011). At the whole-organism level a range of intrinsic and extrinsic factors may elicit variation in CT_{min}, including for example, thermal history, age or sex (Bowler & Terblanche, 2008; Chown & Nicolson, 2004). The onset of chill coma (CT_{min}) and lower lethal temperatures have also been used to compare populations originating from diverse thermal environments (e.g. altitudinal or latitudinal gradients; Klok & Chown, 2003) and thereby infer local adaptation of low temperature performance (e.g. Coyne et al., 1983; Sisodia & Singh, 2010; Alford et al., 2012; Calosi et al., 2012). This idea is however not without controversy. At least four major criticisms and potential confounding factors can be levelled at studies of among-population low temperature tolerance variation. First, in several cases there is a failure to account for, or eliminate, phenotypic plasticity as a confounding factor, as it is well documented that chill coma temperatures can respond readily to short, acute or longer, more chronic thermal history (e.g. Rako & Hoffmann, 2006; Terblanche & Chown, 2006; Cooper et al., 2012). Indeed, in species where chill coma onset or recovery has shown variation among populations, the differences may be equal in magnitude to, or smaller than, plastic trait responses (e.g. Ayrinhac et al., 2004; Hoffmann et al., 2005; Terblanche et al., 2006). Second, when wild populations are compared for low temperature tolerance, potential differences in age-structure among populations are not accounted for. The use of standardized age groups (or the demonstration that age-related variation is not significant) is critical given that thermal tolerance typically varies markedly with age in insects (reviewed in Bowler & Terblanche, 2008). Third, many among-population trait association studies fail to demonstrate that variation in the low temperature tolerance trait examined is under genetic control, despite a wide range of molecular studies which

have sought candidate genes underlying various low temperature traits (e.g Sinclair et al., 2007; Colinet et al., 2010; Overgaard et al., 2010). Finally, in many cases there is a lack of evidence that the low temperature tolerance trait responds, and is subjected to, natural selection (e.g. heritability, directional selection, fitness costs; see discussion in e.g. Coyne et al., 1983). This is of particular interest in species that overwinter, as cold adaptation may help to increase fitness in cold environments (Hoffmann et al., 2003a).

A 'common-garden' or 'common-environment' approach is a widely used method for detection of trait and fitness differences among populations, and which eliminates a range of potential confounding effects (e.g. developmental plasticity, local acclimatization) (Kawecki & Ebert, 2004). This approach also allows linking of the phenotype to the genotype, and potentially also the detection of fitness effects or trade-offs between traits. Common-garden studies can also be used to further inform further molecular work and aid in gene-to-environment integration (Dalziel et al., 2009). Common-garden experiments also provide an ideal situation to undertake traditional quantitative genetic analysis of complex traits through the use of line cross analysis of different populations containing divergent phenotypes (reviewed by Wright, 1968; Lande, 1981; Shaw, 1996). Although more sophisticated methods now exist for calculating and directly mapping gene and single nucleotides (SNPs) influence on traits (Qin et al., 2005; Sinclair et al., 2007), this phenotypic method is particularly helpful for analysing species for which no molecular sequence data is currently available. Although the common-garden approach has long been appreciated, few studies employ this using several populations collected from the wild, and then actively reared under similar, controlled conditions in the laboratory, especially in non-drosophilid study organisms. With a handful of notable exceptions (e.g. Ayres & Scriber, 1994; Ragland & Kingsolver, 2008; Fischer et al., 2010), trait measurement typically occurs under controlled conditions within a few days after wild population collection (Rank et al., 2007; Terblanche et al.,

2008; Pelini et al., 2009) in some cases with a good reason for avoiding other potential confounding factors (e.g. senescence).

Here, I, examine among-population variation in CT_{min} in *E. saccharina*. This species is an agricultural pest of graminaceous crops across a broad geographic range in Africa (Assefa et al., 2006). It exhibits no evidence of overwintering strategies such as diapause in southern Africa (Atkinson & Carnegie, 1978), making it an ideal organism to investigate the link between thermal tolerance and adaptive thermal trait diversification across part of its natural geographic range where a strong thermal cline exists. I, explicitly sought to account for a range of potential confounding effects that might bias the outcome of a comparison amongst populations for CT_{min} variation, including examining and discounting (or controlling for) age, sex, developmental thermal history and short-term thermal acclimation. Specifically, I aimed to determine whether 1) CT_{min} differs among geographic populations and is correlated with local climates, 2) there is evidence of fitness costs of cold tolerance on development time and plastic responses in naturally varying populations and 3) the CT_{min} response is likely to be associated with genotypic variation through genetic crosses and polygene calculation.

3.2 Materials and methods

3.2.1 Study organisms and rearing method

Wild larvae of *E. saccharina* were collected from sugarcane across a range of thermal habitats in South Africa (Figure 3.1A; Table 3.1). A separate study has examined in detail the influence of host plant on CT_{min} and other thermal tolerance traits (Kleynhans et al., 2014), and suggests that while host plant can influence CT_{min} these effects are relatively small (±1.0 °C) and not linked to host plant microclimate variation, especially compared to the magnitude of differences found between

populations here. Each geographic line was founded using 60 – 80 individually field collected larvae (instars: 1-6) and supplemented with larvae from field populations (typically every 1-2 weeks), except for the Malelane colony, which was renewed twice between April and November 2013 because a constant supply of wild specimens was not viable logistically. The laboratory culture used for comparisons in this study was systematically stocked with wild larvae predominantly from the Ginginglovu (29°01'07"S, 31°35'05"E) and Midlands south regions during 2010 to ultimately replace the initial colony in its entirety within 3 generations during 2010. Experiments were conducted approximately 18-24 generations after replacement.

Larvae were maintained on artificial diet in 30 ml vials from field collection until pupation and adult emergence in a quarantine room maintained at 24.0 ± 2.0 °C, 65.0 ± 5.0 % relative humidity. Upon emergence, adult moths were transferred to a common rearing room and placed in tissue-paper lined oviposition boxes with water *ad libitum*. Rearing (following methods outlined in Conlong, 1989; Gillespie, 1993) followed a common-garden approach in which all the populations (wild and laboratory) were reared under similar, thermally-controlled conditions kept at a constant 27.0 ± 2.0 °C, 75.0 ± 5.0 % relative humidity with a 12L:12D hour photoperiod cycle. Whereas the chosen rearing temperature may seem high, many earlier laboratory trials have determined this temperature as being optimal for rearing and maintaining the colony (Kleynhans et al., 2014). Eggs were collected weekly, and placed in a ventilated plastic jar with artificial larval diet. Neonate larvae hatched after 4-6 days and entered the diet medium. They were then transferred individually into vials containing fresh diet medium after roughly twenty days (at instar 3-4 stage). Pupae were removed from these vials and placed into individual cells of replicated multi-cell trays (N = 32 cells per tray) covered with ventilated cling wrap to prevent adult escape. Freshly emerged adult moths were removed from the multi-cell trays daily to track age in 24-h increments.

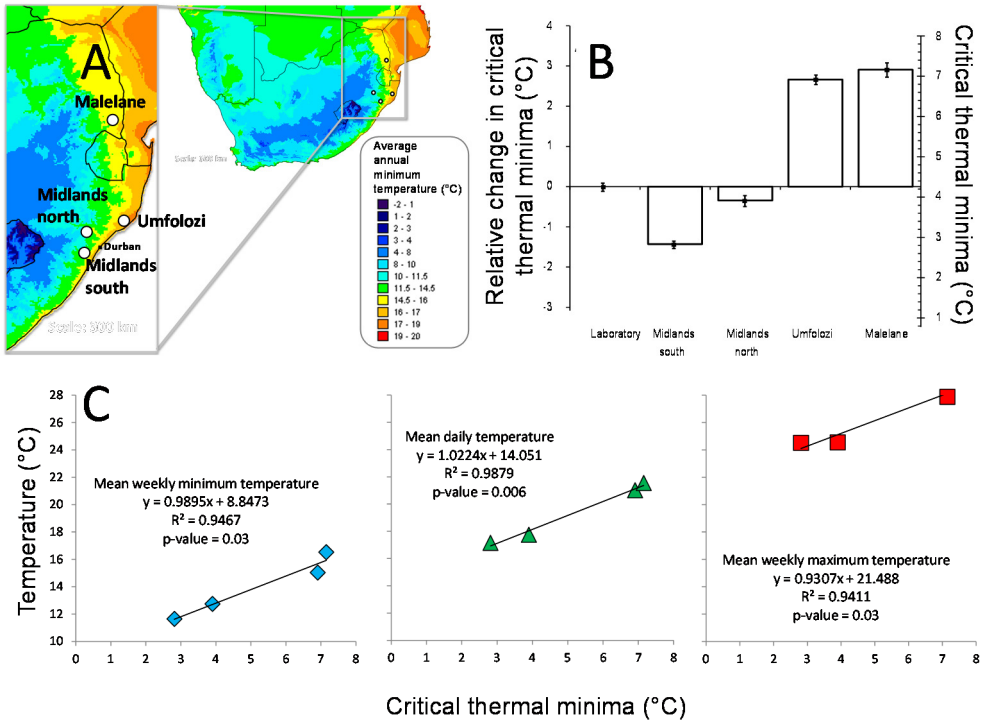

Figure 3.1. A) Geographic collection sites of wild *Eldana saccharina* larvae from sugarcane across a range of thermal habitats in South Africa. B) Differences in chill coma onset temperature (CT_{min}, in °C) between wild geographic lines and laboratory reared moths (left: change in CT_{min} relative to the laboratory population; right: absolute CT_{min} estimates) with means and standard errors (s.e.) plotted. C) Correlations of mean weekly minimum, daily and maximum temperatures with CT_{min} results from four different wild geographic lines.

Table 3.1. Geographic sampling locations for *E. saccharina* larvae. Average estimates of the weekly minimum air temperature (Min.), daily air temperature (Mean) and weekly maximum air temperature (Max.) were obtained from the SASRI weatherweb and downloaded on a weekly time resolution for 2012 from weather stations situated close to sampling locations (typically < 18 km). Lat=latitude, Long=longitude.

Geographic Location	Lat., Long. (deg)	Min.	Mean	Max.
Midlands south[1]	29.9°S, 30.6°E	11.6	17.2	24.5
Midlands north[2]	29.3°S, 30.8°E	12.7	17.7	24.5
Umfolozi	28.5°S, 32.3°E	15.0	21.0	28.4
Malelane	25.5°S, 31.6°E	16.5	21.5	27.9

[1]Mill area = Eston

[2]Mill area = Noodsberg

3.2.2 Chill-coma measurement and analyses

Individual moths (N = 10 per replicate) at two days of age were placed into a double-jacketed insulated chamber ('organ pipes') to measure CT_{min}. Two-day-old moths were used as standard throughout the experiments as age (but not sex) was found to significantly influence CT_{min} estimates (Figure 3.2). The chambers are connected to a fluid circulating, controllable refrigeration bath (Grant GP150-R2, Grant Instruments Inc., UK), filled with ethanol for sub-zero operation. The ethanol was pumped through the organ pipes, circulating around the 11 individual chambers, allowing for controlled cooling of the temperature experienced inside each chamber. A fine, type-T thermocouple (Omega Engineering, Inc., Stamford, CT, Part: 5SC-TT-T-36-36) was inserted into an empty chamber to ensure the desired cooling rate was achieved. The thermocouple was connected, via a multiplexer (USB TC-08 thermocouple data logger, Pico Technology, UK), to a computer acting as a data recorder. Each treatment was replicated three times per geographic line and sex, and in most cases different geographic lines were measured on the same day and order randomized to avoid diurnal effects. After equilibration for 10 minutes at 20.0 °C, temperature was ramped down at a constant ramping rate of 0.1 °C/minute. This is a relatively slow ramping rate but one that is comparable to similar studies (see for example Terblanche et al., 2007). This ramping rate was chosen to be close to the average natural diurnal cooling periods in the Midlands south area (0.02 °C/minute), verified using calibrated thermochron iButton data loggers to record microclimate temperatures at 30 minute sampling frequencies (8-bit Model DS1921, Dallas, TX, USA; 0.5 °C accuracy) at three locations within sugarcane (age 7-9 months).

Moths were prodded gently and regularly with a soft paintbrush until the well-defined CT_{min} endpoint could be observed. For *E. saccharina* adults, CT_{min} was always defined as the temperature at which motor function was lost, shown by onset of twitching and spastic, uncontrolled muscle

movements. Individual moths remained within the chambers during the trials until CT_{min} was reached for all individuals. All individuals were discarded after trials (i.e. not returned to cultures). Statistical analyses were undertaken in R (v. 2.15.1, R Foundation for Statistical Computing, Vienna, Austria; Packages 'MASS' and 'car'). Data were always tested for normality (Shapiro-Wilks test) and over-dispersion (by inspection of residual deviance and degrees of freedom, following Crawley, 2007). The lack of overlap in the 95 % confidence interval was used to identify statistically heterogeneous groups.

To determine whether significant among-population variation in CT_{min} exists, comparisons between the geographic lines and the mass-reared laboratory culture were conducted using a generalized linear model (GLM) (total N = 1080). The mean weekly minimum and maximum temperatures and mean daily temperatures with CT_{min} results from the different lines were used to determine whether the CT_{min} outcomes were correlated with climate and report the R^2 correlation statistic and *P*-values for the linear fit of the line to the CT_{min} and climate data.

To assess the plastic nature of the CT_{min} response, I subjected two-day-old moths (total N = 90) from the Midlands south, laboratory and Malelane populations to 22.0, 27.0 or 32.0 °C for 24 hours. Here I aimed to use a stressor (temperature variation) equal in magnitude both above and below the optimal rearing temperature as it allows for detection of asymmetric responses in CTmin which are sometimes evident (e.g. Rako & Hoffmann, 2006; Terblanche & Chown, 2006). I tested for among-population variation in CT_{min} as a result of short-term temperature variation using a GLM.

I determined the egg to adult duration (total N = 458) and pupal survival (N = 40 per geographic line) under common-garden conditions to investigate whether a fitness or survival cost is a trade-off

during adaptation to cooler environments. These comparisons were made between the Malelane and Midlands south populations using the Wilcoxon-Mann-Whitney rank sum test in R.

To further understand the genetic contribution to trait variation, a series of crosses were undertaken. Wild male and female moths from Malelane (warm population with poorest cold tolerance) were crossed with wild male and female moths from the Midlands south (cold population with greatest cold tolerance), the offspring of which were crossed to generate an F_2 generation. Furthermore, I compared the variation in CTs from the F_1 generation to the variation shown in the F_2 population (total N = 440). Here I focused on obtaining large replicate samples at the individual, rather than the population, level. Focus on the latter would have required lower sample sizes as logistic constraints were already challenging in rearing these numbers in a slowly developing species such as *E. saccharina*. I measured CTs for male and female moths separately to determine whether the thermal tolerance trait may be correlated with genotype (total N = 240). The likelihood of polygenic control of this trait was assessed using phenotypic data for three generations of crosses (parental, F_1 and F_2) and the number of genes calculated following methods outlined by Wright (1968) and Lande (1981) and applied in Shaw (1996). Briefly, variance was partitioned between the different generations to obtain a segregational variance estimate for the F_1 and F_2 generations together with the standard deviation for these measures (Shaw, 1996). These segregation variances for the two generations were then compared to the variance estimates from the two parental populations to obtain an estimate of the number of effective factors (n_E) and their standard error, contributing to the difference in CT_{min} between the two populations (Shaw, 1996). As I did not have sufficient backcross data, I only conducted calculations using equations 1(a and b) and 2(a and b) in Shaw (1996) and used the mean of these estimates to determine the number of polygenes in this trait.

3.3 Results

After rearing four different geographic populations under common-garden conditions, significant among-population variation in CT_{min} was found (Wald χ^2 (4) = 3135.10, $P < 0.0001$; Table 3.2). CT_{min} of the wild geographic lines differed from that of the laboratory culture after rearing the wild geographic lines under common-environment conditions (verified using 95% confidence levels and see Table 3.2). The average CT_{min} estimates were 2.8, 3.9, 6.9 and 7.2 °C for the Midlands south, Midlands North, Umfolozi and Malelane populations respectively. This indicates the order of CT_{min} estimates between populations as Malelane > Laboratory population > Midlands south (Figure 3.1B). Significant positive associations were found between CT_{min} and mean minimum weekly temperature (r^2 = 0.95, P = 0.027), mean daily temperature (r^2 = 0.99, P = 0.006) and mean maximum weekly temperature (r^2 = 0.94, P = 0.030) (Figure 3.1C).

Short-term adult thermal acclimation of the Midlands south, laboratory and Malelane populations had significant effects on CT_{min} (Table 3.3). The Malelane population always showed a higher CT_{min} (i.e. poorer tolerance of cold) in comparison to the other populations, irrespective of acclimation temperature (Figure 3.3A). The magnitude of the acclimation effect was higher for the wild populations in comparison to the laboratory population CT_{min} response. Within wild populations, wither warmer or colder acclimation temperatures resulted in significantly higher CT_{min} compared with an intermediate acclimation temperature, that is, intermediate temperatures generally appear less stressful.

Table 3.2. Summary results from a generalized linear model (with gaussian distribution of errors and an identity link function) of chill-coma onset temperature (°C) among *E. saccharina* populations. Two-day-old adults were measured and compared to the laboratory colony (set as the reference level, or intercept). Model estimates, standard errors (SE), t-values and corresponding *P*–values are given. The residual deviance is 111.76 on 403 degrees freedom and the dispersion parameter is taken to be 0.2773.

Population	Estimate	SE	*t*-value	*P*-value
Intercept	4.25	0.05	84.15	< 0.001
Malelane	2.91	0.09	34.35	< 0.001
Midlands north	-0.34	0.09	-4.03	< 0.001
Midlands south	-1.42	0.09	-16.89	< 0.001
Umfolozi	2.66	0.09	31.30	< 0.001

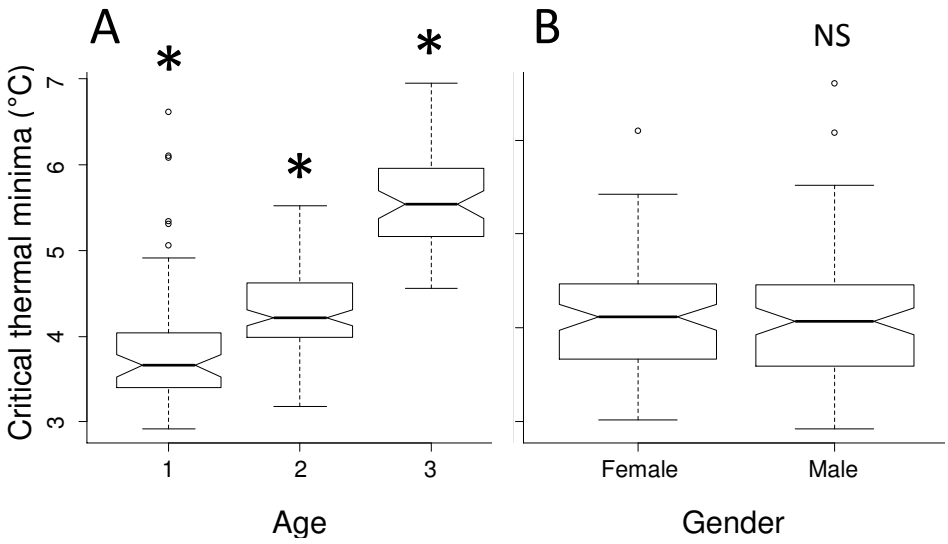

Figure 3.2. Box-plots of variation in critical thermal minima (°C) as a result of A) adult age and B) sex (two-day-old adults). No overlap of triangular box-plot notches were used to identify significant differences in group medians on a 95 % confidence level (Crawley 2007). * Indicates significant differences and NS = non-significant. Preliminary trials were conducted using the laboratory colony to achieve the goals of establishing whether age and sex significantly affected CT_{min} estimates for E. saccharina. One-, two- and three-day-old moths' CT_{min} were measured for males and females separately (N = 30 moths per age and sex, total N = 150). The results of age and sex were analysed and interpreted independently using non-parametric statistics after testing for a significant interaction effect using a generalized linear model (GLZ) with a normal probability distribution of errors and an identity link function. Age data were analysed using a GLZ (specified chi-squared test) and sex data were analysed using the Wilcoxon-Mann-Whitney rank sum test. Age significantly influenced the CT_{min} of *E. saccharina* moths, irrespective of sex (Wald χ^2 (1) = 245.86, $P < 0.0001$) while no significant differences were found between the medians of male and female moth CT_{min} irrespective of their age (W = 3468.50, $P = 0.68$).

Table 3.3. Summary results from a generalized linear model (Gaussian distribution of errors and identity link function) of chill-coma induction temperature of *E. saccharina* (CT_{min} in °C) in response to short term (24 hours at 22.0, 27.0 or 32.0 °C) temperature exposure (Acclimation temperature) in combination with adult moth age (two and three day old moths), explored in the laboratory population. Amongst-population variation in short term temperature exposure responses were compared between the warm populations (grouped Malelane and Umfolozi), cold population (Midlands south) and laboratory stock population. Interaction effects are presented with × and the degrees of freedom (*d.f.*), chi–square (χ^2) statistic, corresponding p–value (*P*) are shown.

Effect	*d.f.*	$\chi 2$	*P*-value
Age	1	481.02	<0.0001
Acclimation temperature	2	175.98	<0.0001
Age × Acclimation temperature	2	49.72	<0.0001
Population	3	2960.6	<0.0001
Acclimation temperature	2	1315.7	<0.0001
Population × Acclimation temperature	6	1299.8	<0.0001

There was a significant fitness trade-off associated with living in the cooler environment (i.e. in the population showing the lowest CT_{min}). The geographic lines that were more cold tolerant (Midlands south: low CT_{min}) took longer to reach the adult life-stage under similar rearing conditions, than the Malelane population that had a higher CT_{min} response (Figure 3.4A, Wald χ^2 (1) = 12.920, $P < 0.001$). However, the benefit to being more cold tolerant is illustrated in the Midlands south population, which had a significantly higher survival than the Malelane population under common-garden conditions (Figure 3.4B, Wald χ^2 (1) = 8.930, $P = 0.003$).

The results of the crosses between most and least cold-tolerant populations reflected an intermediate phenotype in comparisons to the phenotypic responses of the two parent populations, with the CT_{min} estimates for the F_1 generation lying closer to values of the Malelane population than Midlands south. This suggests Mendelian inheritance for CT_{min} in the observed field population that is likely under polygenic control. Further evidence for this is seen in the wider frequency distribution of the F_2 generation (Figure 3.3C). The number of genes (n_E) underlying the CT_{min} trait was calculated from the observed phenotype distributions using equations 1 and 2 in Shaw (1996). The mean of the two n_E calculation methods was 4.09 ± 0.06 (± s.e.). There was also a significant interaction effect of population and sex on CT_{min} of the F_1 generation of crosses between the most cold tolerant (Midlands south) and the least cold tolerant (Malelane) populations (Figure 3.3B).

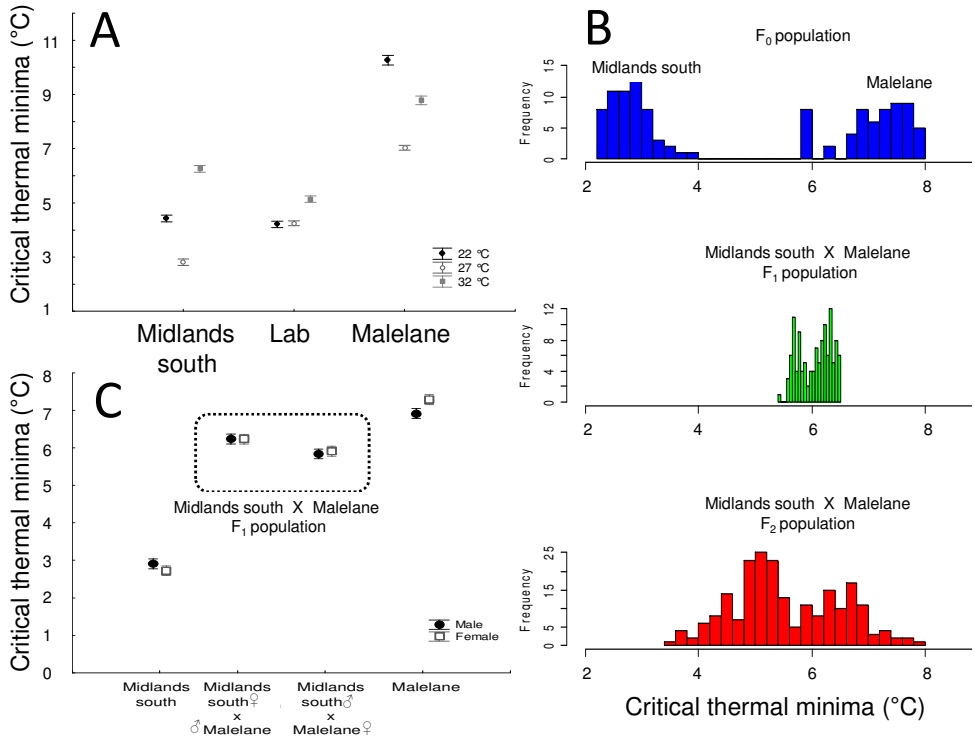

Figure 3.3. A) CT_{min} responses of two-day old moths from the Midlands south, laboratory and Malelane populations acclimated for 24 hours at 22, 27 or 32 °C Vertical bars denote 95% confidence limits. B) A comparison of the variation in CT_{min} from the parental (F_0: Malelane [warm population with low cold tolerance] and Midlands south [cold population with higher cold tolerance]) populations, their crosses (F_1) and a further F_1 x F_1 cross (F_2). C) CT_{min} estimates of F_0 and F_1 by sex following crosses in both directions i.e. Female Malelane x Male Midlands south and *vice versa*. Vertical bars denote 95% confidence limits.

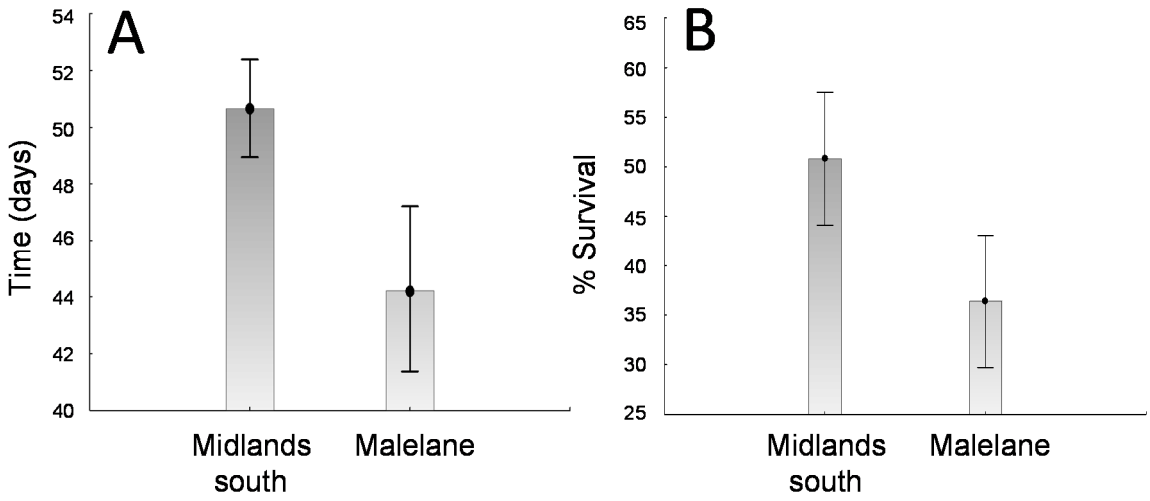

Figure 3.4. A) Mean egg to adult development time and B) pupal survival of the Malelane and Midlands south populations when reared under common-garden conditions. Vertical bars denote 95% confidence limits.

3.4 Conclusion

Temperature influences the basic physiological components that are fundamental to understanding the responses of insects in the wild. Tolerance to low temperatures is generally considered to limit distributions of insect species, particularly in northern hemisphere species. These organisms, however, also exhibit a number of strategies for coping with and avoiding extreme cold conditions, such as freeze-tolerance and diapause/overwintering (mechanisms reviewed in Denlinger, 2002; Chown & Nicolson, 2004). For species that must endure cold conditions, particularly in the southern Hemisphere, there is poorer understanding of the association between cold tolerance limits and environmental variation (Sinclair & Chown, 2005). In particular, inter-population variation in CT_{min} is poorly understood, but given the patterns found at broad geographic and evolutionary scales (e.g. Klok & Chown, 2003; Kimura, 2004; Kellermann et al., 2009; Calosi et al., 2012) it is presumed reasonable that this trait should respond to natural selection for local climate. Further,

species with broad distributions have higher genetic variation for traits that might limit distributions of more narrowly distributed species (e.g. Hoffmann et al., 2003b; Kellermann et al., 2009). Yet evidence for genetic adaptation of thermal tolerance traits in natural populations is important and not often shown for non-model laboratory species (but see e.g. Ayres & Scriber, 1994; Klok & Chown, 2003).

Here, using wild populations of the sugarcane stalk borer *E. saccharina* in South Africa as a model system, I explicitly account for several of the most prevalent potential draw-backs of low temperature stress resistance studies, including the failure to account for plastic responses, thermal history, sex, age and overwintering effects. Plastic effects were eliminated by rearing geographic lines under similar, controlled conditions for at least two to three generations prior to experiments. By accounting for these potential confounding factors, I were able to demonstrate that variation in CT_{min} is under genetic control and provide evidence that CT_{min} responds to, and is likely subjected to selection in the wild. I furthermore correlated the differences in geographic lines with local climates to test the theory that genetic change may facilitate increased field fitness after temporal adaptation to novel environments. CT_{min} from the different populations were significantly positively correlated with minimum, mean and maximum local temperature. The warm population showed poor cold tolerance after thermal acclimation and both the warm and cold wild populations showed an increased variation in CT_{min} in comparison to the laboratory population, indicating local variation in plastic responses for this trait. I conclude that sufficient selective potential for physiological adaptation likely facilitates the invasion success of *E. saccharina* into novel environments, evidenced in the clear genetic architecture underlying CT_{min} trait variation for this species.

The evidence of prior selection in response to the environment between populations of *E. saccharina* appears to have significant physiological costs, resulting in a major increase in development time of individuals from the more cold-tolerant Midlands south population. As this phenological trade-off persists after several generations under common-garden experiments, it is clearly not simply the result of plastic changes. It is not yet possible however to determine if the slower development time of the more cold-tolerant Midlands south population is the result of a cost of CT_{min} adaptation, or a general life-history trade-off to maximise survival under the colder conditions of this habitat. Irrespective, the persistence of this trait further highlights the importance of improved thermal tolerance for the Midlands south population, as such costs i.e. slow development, would be unlikely to persist without consistent selection (see discussion in Frazier et al., 2006). This is also evident in the plastic responses of this species, controlled for by the common-garden design but later investigated following short-term thermal history manipulation (acclimation; Rako & Hoffmann, 2006; Terblanche & Chown, 2006). Adult acclimation was also found to affect CT_{min} estimates in *E. saccharina* significantly. However, the magnitude of within-population plastic responses to acclimation was much smaller than the between-population historical differences, despite rearing under common-garden conditions (*contra* Ayrinhac et al., 2004; Hoffmann et al., 2005; Terblanche et al., 2006), although it may partly reflect differing methodology to induce plasticity among these studies, as a result of the acclimation regime. This, together with the significant reduction in plasticity in the laboratory-reared population, indicates a benefit for plastic responses to acclimation in natural populations, which is likely to be costly from a fitness perspective, and subsequently lost when no longer selected for (as seen in the laboratory population). It is therefore likely that higher cold tolerance (lower CT_{min}) and plasticity in response to acclimation is an acquired trait for this species and one that may be relevant for predicting this species distribution and future invasive potential.

I chose to rear the *E. saccharina* populations under common conditions using temperatures that appear relatively warm compared to the natural environmental conditions experienced at these localities (Table 3.1). This 27.0 °C rearing temperature was found from previous experiments to maximise survival and fitness of this species under laboratory conditions; however, it may prove to be a more stressful environment for the populations from colder environments i.e. Midlands south. Weather data averaged over a year (or a week) taken from a climate station 2m above ground may however be poorly related to the thermal microenvironment preferred and used by an insect species such as *E. saccharina* (see e.g. Andrew et al., 2013; Potter et al., 2013). The slower development time of the Midlands south population may be evidence that these rearing temperatures are potentially stressful. However, the greater overall survival of Midlands south individuals, particularly in comparison to the Malelane population that experiences warmer conditions, indicates to us that this is unlikely to be the case. Future investigations of this species could consider developmental (and other forms of) acclimation effects in further detail. These were outside the scope of this particular study.

I have also found evidence for a genetic component to CT_{min} trait variation, suggesting that local environmental adaptation has likely taken place amongst these *E. saccharina* populations. The similarity of the F_1 generation CT_{min} estimates to those of the Malelane parental population, together with the development time costs and reduced CT_{min} estimates in the laboratory population indicate that the improved cold tolerance in the Malelane and Midlands south populations, are acquired traits. The bimodal distribution of the frequency distribution of the F_2 generation suggests that the genetic architecture underlying CT_{min} variation is probably representing four key genes. This is in accordance with literature on *Drosophila* spp., with four key candidate genes originally identified via meta-analysis (*Dca/Smp-30, Fst, hsr-omega* and *drosomycin*; Hoffmann et al., 2003c). However, more recent studies that have investigated these genes, plus highlighted other possible

genes associated with cold tolerance assays in *Drosophila*, have found equivocal evidence for their association. Using qRT-PCR, Sinclair et al., (2007) investigated gene expression of *Fst*, *desat-2*, *Smp-30, hsp23* and *hsp70* following exposure and recovery from cold shock in *D. melanogaster* Fallén (Diptera: Drosophilidae). They found no evidence for upregulation of any candidate genes during cold exposure but some implication of *Fst* and *hsp70* in the recovery phase (Sinclair et al., 2007), in agreement with studies of *hsps* after prolonged exposure to zero °C (Colinet et al., 2010) and expression of *Fst* in lines following cold shock at zero °C (Goto, 2001) in *D. melanogaster*. Population studies of gene polymorphisms in *Fst* in this species along the east coast of Australia found strong evidence of clinal association in different alleles of this gene, but no association with 3 different estimates of cold tolerance associated with chill coma recovery (Hoffmann et al., 2012). It is likely, therefore, that the candidate genes identified for cold tolerance in insects are more important for recovery from cold exposure rather than directly associated with cold tolerance (Sinclair et al., 2007; Sinclair et al., 2012).

The majority of studies to date assay cold tolerance using some measure of chill-coma recovery or rapid cold hardening using the model organism, *D. melanogaster* (see review in e.g. Sinclair et al., 2012). Very few have considered the mechanisms underlying CT_{min} (onset of failure) responses, either through association in natural populations or via a candidate gene approach. Ransberry et al., (2011) examined the correlation between phenotypic plastic responses in CCRT and CT_{min} assays in *D. melanogaster* and found evidence for association in plastic responses in CCRT and CT_{min} in a single population. However this was not maintained across all acclimation temperatures, indicating incomplete overlap between the mechanisms underlying plastic responses in both estimates (Ransberry et al., 2011). The similarity between the mechanisms underlying the basal trait responses of CCRT and CT_{min} has received little attention. The lack of congruence between different studies of the same model organism plus some level of decoupling of the plasticity

underlying these traits suggests that a candidate gene approach may not be sufficient for investigating genetic processes underlying CT_{min} as a trait. Further, studies of *D. melanogaster* regularly show that the thermal tolerance and plasticity estimates of this species do not necessarily reflect that of species within the same genera, let alone different insect species (e.g. Nyamukondiwa et al., 2011).

To better understand the mechanisms underlying the CT_{min} response and its relevance in natural populations, it may be necessary to investigate this trait in non-model organisms, as presented here. Moreover, the apparent fitness costs of maintaining improved cold tolerance and plasticity for CT_{min} in natural populations of *E. saccharina* indicate that this trait may have more importance in natural populations than has previously been assumed. Following the results of this study, *E. saccharina*, with evidence of recent adaptive changes in this trait and relatively simple genetic architecture, may be an ideal study organism to investigate these mechanism(s) further. A full-genome screening approach of populations with contrasting CT_{min} values, relative to laboratory controls, would add further knowledge and improve the value of this as a model system for understanding evolved chill coma variation in insects.

3.5 References

Addo-Bediako, A., Chown, S. L. & Gaston, K. J. (2000) Thermal tolerance, climatic variability and latitude. Proceedings of the Royal Society B: Biological Sciences 267: 739–745.

Alford, L., Blackburn, T. M. & Bale, J. S. (2012) Effects of acclimation and latitude on the activity thresholds of the aphid *Myzus persicae* in Europe. Journal of Applied Entomology 136: 332–346.

Anderson, R. L. & Mutchmor, J. A. (1968) Temperature acclimation and its influence on electrical activity of nervous system in three species of cockroaches. Journal of Insect Physiology 14: 243–251.

Anderson, A. R., Hoffmann, A. A. & McKechnie, S. W. (2005) Response to selection for rapid chill-coma recovery in *Drosophila melanogaster*: physiology and life-history traits. Genetic Research 85: 15–22.

Andrew, N. R., Hart, R. A., Jung, M. P., Hemmings, Z. & Terblanche, J. S. (2013) Can temperate insects rake the heat? A case study of the physiological and behavioural responses in a common ant *Iridomyrmex purpureus* (Formicidae) with potential climate change. Journal of Insect Physiology 59: 870–880.

Assefa, Y., Mitchell, A. & Conlong, D. E. (2006) Phylogeography of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Annales de la Société Entomologique de France 42: 331–337.

Atkinson, P. R. & Carnegie, A. J. M. (1978) Population dynamics of the sugarcane borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae), in Natal, South Africa. Bulletin of Entomological Research 79: 61–80.

Ayres, M. P. & Scriber, J. M. (1994) Local adaptation to regional climates in *Papilio canadenis* (Lepidoptera: Papilionidae). Ecological Monographs 64: 465–482.

Ayrinhac, A., Debat, V., Gibert, P., Kister, A-G., Legout, H., Moreteau, B., Vergilino, R. & David, J. R. (2004) Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. Functional Ecology 18: 700–706.

Bijlsma, R. & Loeschcke, V. (2005) Environmental stress, adaptation and evolution: an overview. Journal of Evolutionary Biology 18: 744–749.

Bowler, K. & Terblanche, J. S. (2008) Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? Biological Reviews 83: 339–355.

Calosi, P., Bilton, D. T., Spicer, J. I., Verberk, W. C. E. P., Atfield, A. & Garland, T. (2012) The comparative biology of diving in two genera of European Dytiscidae (Coleoptera). Journal of Evolutionary Biology 25: 329–341.

Chown, S. L. & Nicolson, S. W. (2004) Insect physiological ecology. Mechanisms and patterns. Oxford University Press, Oxford, U.K.

Colinet, H., Lee, S. F. & Hoffmann, A. A. (2010) Knocking down expression of Hsp22 and Hsp23 by RNA interference affects recovery from chill coma in *Drosophila melanogaster*. Journal of Experimental Biology 213: 4146–4150.

Conlong, D. E. (1989) Technique development for mass rearing. Proceedings of the first insect rearing workshop held during the seventh national congress of the entomological society of southern Africa. Pp. 10–14.

Cooper, B. S., Tharp II, J. M., Jernberg, I. I. & Angilletta, M. J. (2012) Developmental plasticity of thermal tolerances in temperate and subtropical populations of *Drosophila melanogaster*. Journal of Thermal Biology 37: 211–216.

Coyne, J. A., Bundgaard, J. & Prout, T. (1983) Geographic variation of tolerance to environmental stress in *Drosophila pseudoobscura*. The American Naturalist 122: 474–488.

Crawley, M. J. 2007. *The R book.* John Wiley & Sons Ltd, Chichester, U.K.

Dalziel, A. C., Rogers, S. M. & Schulte, P. M. (2009) Linking genotypes to phenotypes and fitness: how mechanistic biology can inform molecular ecology. Molecular Ecology 18: 4997–5017.

David, J. R., Gibert, P., Legout, H., Pétavy, G., Capy, P. & Moreteau, B. (2005) Isofemale lines in Drosophila: an empirical approach to quantitative trait analysis in natural populations. Heredity 94: 3–12.

Denlinger, D. L. (2002) Regulation of diapause. Annual Review of Entomology 47: 93–122.

Fischer, K., Dierks, A., Franke, K., Geister, T. L., Liszka, M., Winter, S. & Pflicke, C. (2010) Environmental effects on temperature stress resistance in the tropical butterfly *Bicyclus Anynana*. PLoS ONE 5: e15284.

Frazier, M. R., Huey, R. B. & Berrigan, B. (2006) Thermodynamics constraints the evolution of insect population growth rates: "Warmer is better". The American Naturalist 168: 512–520.

Ghalambor, C. K., McKay, J. K., Carroll, S. P. & Reznick, D. N. (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21: 394–407.

Gillespie, D. (1993) Development of mass-rearing methods for the sugarcane borer *Eldana saccharina* (Lepidoptera: Pyralidae) ii: Diet gelling agents. Proceedings of the South African Sugar Technologists' Association 67: 127–129.

Goller, F. & Esch, H. (1990) Comparative study of chill-coma temperature and muscle potentials in insect flight muscles. Journal of Experimental Biology 150: 221–231.

Goto, S. G. (2001) A novel gene that is up-regulated during recovery from cold shock in *Drosophila melanogaster*. Gene 270: 259–264.

Hazell, S. P. & Bale, J. S. (2011) Low temperature thresholds: are chill coma and CT_{min} synonymous? Journal of Insect Physiology 57: 1085–1089.

Hill, M. P., Hoffmann, A. A., Macfadyen, S., Umina, P. A. & Elith, J. (2012) Understanding niche shifts: using current and historical data to model the invasive redlegged earth mite *Halotydeus destructor*. Diversity and Distributions 18: 191–203.

Hill, M. P., Chown, S. L. & Hoffmann, A. A. (2013) A predicted niche shift corresponds with increased thermal resistance in an invasive mite, *Halotydeus destructor*. Global Ecology and Biogeography 22: 942–951.

Hoffmann, A. A., Anderson, A. & Hallas, R. (2002) Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. Ecology Letters 5: 614–618.

Hoffmann, A. A., Scott, M., Partridge, L. & Hallas, R. (2003a) Overwintering in *Drosophila melanogaster*: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. Journal of Evolutionary Biology 16: 614–623.

Hoffmann, A. A., Hallas, R. J., Dean, J. A. & Schiffer, M. (2003b) Low potential for climatic stress adaptation in a rainforest *Drosophila* species. Science 301: 100–102.

Hoffmann, A. A., Sørensen, J. G. & Loeschcke, V. (2003c) Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. Journal of Thermal Biology 28: 175–216.

Hoffmann, A. A., Shirriffs, J. & Scott, M. (2005) Relative importance of plastic *vs* genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. Functional Ecology 19: 222–227.

Hoffmann, A. A., Blacket, M. J., McKechnie, S. W., Rako, L., Schiffer, M., Rane, R. V., Good, R. T., Robin, C. & Lee, S. F. (2012) A proline repeat polymorphism of the *Frost* gene of *Drosophila melanogaster* showing clinal variation but not associated with cold resistance. Insect Molecular Biology 21: 437–445.

Hoffmann, A. A., Chown, S. L. & Clusella-Trullas, S. (2013) Upper thermal limits in terrestrial ectotherms: how constrained are they? Functional Ecology 27: 934–949.

Huey, R. B. (2010) Evolutionary physiology of insect thermal adaptation to cold environments. In: Low temperature biology of insects (Denlinger, D. L. & Lee, R. E. eds.), pp. 223–241. Cambridge University Press, Cambridge, U.K.

Huey, R. B. & Stevenson, R. D. (1979) Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. American Zoologist 19: 357–366.

Jenkins, N. L. & Hoffmann, A. A. (2001) Distribution of *Drosophila serrata* Malloch (Diptera: Drosophilidae) in Australia with particular reference to the southern border. Australian Journal of Entomology 40: 41–48.

Kawecki, T. J. & Ebert, D. (2004) Conceptual issues in local adaptation. Ecology Letters **7**: 1225–1241.

Kellermann, V., van Heerwaarden, B., Sgrò, C. M. & Hoffmann, A. A. (2009) Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. Science 325: 1244–1246.

Kimura, M. T. (2004) Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. Oecologia 140: 442–449.

Kingsolver, J. G. (1983) Thermoregulation and flight in Colias butterflies – elevational patterns and mechanistic limitations. Ecology 64: 53–545.

Kingsolver, J. G. (2009) The well-temperatured biologist. American Naturalist 174: 755–768.

Kingsolver, J. G., Ragland, G. J. & Diamond, S. E. (2009) Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. Evolution 63: 537–541.

Kleynhans, E., Conlong, D. E. & Terblanche, J. S. 2014. Host plant-related variation in thermal tolerance of *Eldana saccharina* (Lepidoptera: Pyralidae). Entomologia Experimentalis et Applicata 150: 113–122.

Klok, C. J. & Chown, S. L. (2003) Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. Biological Journal of the Linnean Society 78: 401–414.

Lande, R. (1981) The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99: 541–553.

MacMillan, H. A. & Sinclair, B. J. (2011) Mechanisms underlying insect chill-coma. Journal of Insect Physiology 57: 12–20.

Nyamukondiwa, C., Terblanche, J. S., Marshall, K. E. & Sinclair, B. J. (2011) Basal cold but not heat tolerance constrains plasticity among *Drosophila* species (Diptera: Drosophilidae). Journal of Evolutionary Biology 24: 1927–1938.

Overgaard, J., Hoffmann, A. A. & Kristensen, T. N. (2011) Assessing population and environmental effects on thermal resistance in *Drosophila melanogaster* using ecologically relevant assays. Journal of Thermal Biology 36: 409–416.

Overgaard, J., Sørensen, J. G., Jensen, L. T., Loeschcke, V. & Kristensen, T. N. (2010) Field tests reveal genetic variation for performance at low temperatures in *Drosophila melanogaster*. Functional Ecology 24: 186–195.

Pelini, S. L., Dzurisin, J. D. K., Prior, K. M., Williams, C. M., Marsico, T. D., Sinclair, B. J. & Hellmann, J. J. (2009) Translocation experiments with butterflies reveal limits to enhancement of poleward populations under climate change. Proceedings of the National Academy of Sciences of the United States of America 106: 11160–11165.

Potter, K. A., Woods, H. A. & Pincebourde, S. (2013) Microclimatic changes in global change biology. Global Change Biology 19: 2932–2939.

Qin, W., Neal, S. J., Robertson, R. M., Westwood, J. T. & Walker, V. K. (2005) Cold hardening and transcriptional change in *Drosophila melanogaster*. Insect Molecular Biology 14: 607–613.

R Development Core Team. (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org.

Ragland, G. J. & Kingsolver, J. G. (2008) Evolution of thermotolerance in seasonal environments: the effects of annual temperature variation and life-history timing in *Wyeomyia smithii*. Evolution 62: 1345–1357.

Rako, L. & Hoffmann, A. A. (2006) Complexity of the cold acclimation response in *Drosophila melanogaster*. Journal of Insect Physiology 52: 94–104.

Rako, L., Blacket, M. J., McKechnie, S. W. & Hoffmann, A. A. (2007) Candidate genes and thermal phenotypes: identifying ecologically important genetic variation for thermotolerance in the Australian *Drosophila melanogaster* cline. Molecular Ecology 16: 2948–2957.

Rank, N. E., Bruce, D. A., McMillan, D. M., Barclay, C. & Dahlhoff, E. P. (2007) Phosphoglucose isomerase genotype affects running speed and heat shock protein expression after exposure to extreme temperatures in a montane willow beetle. Journal of Experimental Biology 210: 750–764.

Ransberry, V. E., MacMillan, H. A. & Sinclair, B. J. (2011) The relationship between chill-coma onset and recovery at the extremes of the thermal window of *Drosophila melanogaster*. Physiological and Biochemical Ecology 84: 553–559.

Shaw, K. L. (1996) Polygenic inheritance of a behavioural phenotype: interspecific genetics of song in the Hawaiian cricket genus *Laupala*. Evolution 50: 256–266.

Sinclair, B. J. (2001) Biologically relevant environmental data: macros to make the most of microclimate recordings. CryoLetters 22: 125–134.

Sinclair, B. J. & Chown, S. L. (2005) Climatic variability and hemispheric differences in insect cold tolerance: support from southern Africa. Functional Ecology 19: 214–221.

Sinclair, B. J., Gibbs, A. G. & Roberts, S. P. (2007) Gene transcription during exposure to, and recovery from, cold and desiccation stress in *Drosophila melanogaster*. Insect Molecular Biology 16: 435–443.

Sinclair, B. J., Williams, C. M. & Terblanche, J. S. (2012) Variation in thermal performance among insect populations. Physiological and Biochemical Zoology 85: 594–606.

Sisodia, S. & Singh, B. N. (2010) Influence of developmental temperature on cold shock and chill coma recovery in *Drosophila ananassae*: acclimation and latitudinal variations among Indian populations. Journal of Thermal Biology 35: 117–124.

Terblanche, J. S. & Chown, S. L. (2006) The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera, Glossinidae). Journal of Experimental Biology 209: 1064–1073.

Terblanche, J. S., Klok, C. J., Krafsur, E. S. & Chown, S. L. (2006) Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modelling. The American Journal of Tropical Medicine and Hygiene 74: 786–794.

Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C. & Chown, S. L. (2007) Critical thermal limits depend on methodological context. Proceedings of the Royal Society B: Biological Sciences 274: 2935–2942.

Terblanche, J. S., Clusella-Trullas, S., Deere, J. A. & Chown, S. L. (2008) Thermal tolerance in a south-east African population of tsetse fly *Glossina pallidipes* (Diptera, Glossinidae): implications for forecasting climate change impacts. Journal of Insect Physiology 54: 114–127.

Terblanche, J. S., Hoffmann, A. A., Mitchell, K. A., Rako, L., le Roux, P. C. & Chown, S. L. (2011) Ecologically relevant measures of tolerance to potentially lethal temperatures. Journal of Experimental Biology 214: 3713–3725.

Wright, S. (1968) *Evolution and the genetics of populations. Vol. I. Genetic and biometric foundations.*

Chapter 4

Direct and indirect effects of development temperature on adult water balance traits of *Eldana saccharina* (Lepidoptera: Pyralidae)

Published as Kleynhans, E., Conlong, D. E. & Terblanche, J. S. (2014) Direct and indirect effects of development and temperature on adult water balance traits of *Eldana saccharina* (Lepidoptera: Pyralidae). *Journal of Insect Physiology* 68: 69-75.

4.1 Introduction

Terrestrial insects may be dramatically influenced by water availability in their environment, in part owing to their small body size and high surface area-to-volume relationship (Hadley, 1994). Environmental water availability, and an insect's ability to manage its water loss rate or withstand losing water, can have a direct impact on population abundances and geographic distribution, through activity and survival times (reviewed in Hadley, 1994 and see e.g. Kellermann et al., 2009; Benoit et al., 2010; Chown et al., 2011; Overgaard et al., 2014). Death may occur upon exhaustion of either water or lipid reserves from the insect body (Hoffmann & Harshman, 1999; Marron et al., 2003). Essentially survival time under low humidity conditions of an insect is a function of how much body water the insect has, the rate at which it is lost, and how much water it can withstand losing (Hadley, 1994; Chown et al., 2011).

Simulations of forecast climate change, with predictions of increasing frequency and severity of droughts (e.g. Easterling et al., 2000; Fortain et al., 2010), suggest that terrestrial insects could be affected by changes in their evaporative water loss rates and changing environmental moisture availability (Chown et al., 2011). However, climate change is likely to involve concurrent changes in both moisture availability (e.g. relative humidity, precipitation) and ambient temperature, thus emphasizing the need to understand both aspects of these two abiotic variables on insect physiological responses and water balance at various time-scales (Chown et al., 2011; Kleynhans & Terblanche, 2011).

Water loss, as a result of respiration and cuticular transpiration, pose significant challenges to terrestrial insects in novel or changing environments (Woods & Smith, 2010; Chown et al., 2011). Furthermore, insect are susceptible to increased respiratory water loss with increasing temperature

(e.g. Terblanche et al., 2010) since respiration rates follow an exponential pattern and are generally doubled for every 10.0 °C increase in temperature. This means that at higher ambient temperatures, even small changes in temperature can result in large differences in metabolic rate, and presumably also respiratory water loss (Addo-Bediako et al., 2002; Dillon et al., 2010; Kearney, 2012). Although the temperature dependence of biological rates is well accepted and typically follows an exponential relationship (e.g. Dell et al., 2011), any general expectation for what the impacts of prior thermal history might be on these rates is far more contentious. It is clear that prior temperature or relative humidity exposure can have a marked effect on subsequent water loss rates in insects via physiological adjustments (Hoffmann et al., 2005; Parkash et al., 2005, but see Gibbs et al., 2003; Gray & Bradley, 2005; Terblanche et al., 2005; Bedick et al., 2006;).

The influence of rearing temperature on body size is generally well examined in insects (e.g. Gaston & Chown, 2013). By contrast, the influence of rearing temperature on various traits of insect water balance and related traits associated with body size variation are largely unclear (see reviews and discussion in e.g. Leinaas et al., 2009; Terblanche & Kleynhans, 2009). Two alternative hypotheses can be proposed for responses of water balance physiology to rearing temperature. First, prior thermal history may pre-condition individuals to be more sparing in their water consumption at a given temperature upon subsequent exposure, or alternatively, prior exposure may not be stressful and thus relax constraints on water economy leading to more frivolous use of water at a later stage. Estimates of body water content, and rates of water loss, and to a lesser extent, critical body water contents at time of death (defined as BWC_{CRIT} here), have all enjoyed considerable attention to date (e.g. Hadley, 1994; Addo-Bediako et al., 2001; Bazinet et al., 2010). It is therefore surprising that the influence of rearing temperature on these various components of water balance, and what the net outcome of rearing temperature is for survival under dehydrating conditions, has not been well examined (Leinaas et al., 2009). Regardless, the magnitude and direction of phenotypic plasticity in

response to developmental acclimation is important as this forms a critical component of understanding insect population dynamics under variable field conditions (Fallis et al., 2014; Kleynhans et al., 2014a).

The ability to acclimate, and thus alter phenotype-related physiology (Huey et al., 1999), or adapt to environmental change within a short time frame have to be considered and interpreted within the functional and genetic constraints of an insect (Cooper et al., 2010). Acclimation of water balance physiology might thus enhance fitness, but it might also come at a cost or lead to sub-optimal trait responses (see discussions in Hoffmann, 1995; Huey & Berrigan, 1996; Deere & Chown, 2006; Terblanche & Kleynhans, 2009). The consequence of rearing history (developmental plasticity) on the adult life stage is however, not often separated from within-life stage consequences of environmental variability (but see Terblanche & Chown, 2006). Phenotypic plasticity of physiological traits can be beneficial for survival and evolutionary fitness (Piersma & van Gils, 2011; Kleynhans et al., 2014a).

Depending on temperature and diet, the life-cycle of *E. saccharina* lasts for 2 – 3 months (Girling, 1978; Way, 1995). Whether the life stages of *E. saccharina* can adjust readily to enhance their water-balance related physiological performance within or across life-stages has not been explored previously. Here I assessed the two major alternative hypotheses for the impacts of rearing temperature on water balance-related traits (e.g. hydration and water economy) on the adult life stage of *E. saccharina* by exposing immature stages of this species to different rearing temperatures both above and below optimum conditions, and then measuring the resultant adult physiological performance (water loss rates, time to death) and water-balance related traits (body size, water content). I also sought to assess what traits varied in response to immature stage rearing

temperature, and what the net outcome thereof might be for survival of desiccating conditions as adults.

4.2 Materials and methods

Developmental acclimation effects of temperature on male and female *E. saccharina* WLR, BWC and time to death were tested individually following larval to adult rearing at constant 20.0 ± 1.0 °C, 25.0 ± 1.0 °C or 30.0 ± 1.0 °C and 76.0 ± 5.0 % relative humidity (found to be the optimum relative humidity range for laboratory rearing). Humidity was controlled using a saturated sodium chloride salt solution (~ 360 g NaCl in 1 L distilled water at 20 °C), placed on the bottom of 50 L incubators used during immature stage acclimation (following Winston & Bates, 1960). In the wild, development of *E. saccharina* continues throughout the year, resulting in multivoltine populations. Average temperatures for the warmest quarter (average summer temperatures) of the year were 20.0 °C in the southern Midlands of KwaZulu-Natal of South Africa (29.9 °S, 30.6 °E), 25.3 °C for Mpumalanga, South Africa (25.5 °S, 31.6 °E) and 30.1 °C in Kenya (1.5 °S, 36.6 °E). *Eldana saccharina* occurs naturally in all these areas, but their biotypes are different (Assefa et al., 2005). I chose three developmental acclimation temperatures representing those experienced by these wild populations during the warmest quarter of the year in their relevant areas. I used long term average temperatures from the WorldClim database (1970 – 2000 data; Hijmans et al., 2000) to determine the corresponding site- and rearing temperatures.

The effects of immature stage rearing temperature (i.e. acclimation) on water balance-related traits on two-day old adult moths were estimated using gravimetric methods (see description below). I specifically chose this age since activity and mating generally occurs between 24 – 48 hours after adult emergence (Dick, 1945), female egg-laying peak on the second and third night after

emergence (Dick, 1945; Way et al., 1994), and most importantly, two-day-old male moths are used for sterile insect release programs. Rearing followed the methods outlined in Conlong (1989), Gillespie (1993) and Kleynhans et al., (2014b). In brief, the egg stage developed at 24.0 ± 2.0 °C. Larvae that emerged from N > 1200 eggs were transferred into nine 500ml jars, each containing eight 30ml vials with 10ml artificial diet medium each (for further information on the artificial diet see Gillespie, 1993). The jars with the larvae were placed at the three respective developmental temperatures (three replicated jars per treatment). After ~260 heat units (see Way, 1995) third instar larvae were transferred individually into 30ml vials containing 10ml artificial diet. The vials were returned to the respective acclimation temperatures in their respective incubators. Pupae, when formed, were removed from the vials and placed into individual cells of replicated multi-cell trays within 24h of pupation. The trays were kept in the same incubators as the larvae, to complete development at the three respective temperatures. Freshly emerged adult moths were removed from the multi-cell trays daily in order to track age (in days) and sex. Moths (at least 30 males and 30 females per developmental temperature) were weighed individually to determine body mass, WLR and BWC. The initial body mass, minus the body mass at time of death, divided by the time to death was used to calculate WLR. Following the measurement of adult body mass at death, moths were baked dry following death, at ~ 80 °C for 72 h until a constant dry body mass was reached. I tested for the effect of acclimation and sex on BWC expressed as a proportion of body mass (initial weight) by subtracting dry body mass from initial body mass (Hadley, 1994). The $BWC_{INITIAL}$ was estimated as the difference between dry body mass and initial body mass and likely represents the hydrated condition. BWC_{CRIT} was calculated as the difference between dry body mass and the body mass at death. To determine the survival time at < 5 % relative humidity, moths were placed in individually labelled vials on silica gel to obtain the desired desiccating conditions. Time to death, of male and female moths, at 25.0 °C, < 5.0 % relative humidity was scored hourly for each acclimation group. Moths were baked dry upon death to measure BWC.

Adult body masses were obtained individually on an electronic microbalance to 0.1 mg (Mettler Toledo ML54; Mettler-Toledo, Greifensee, Switzerland). All statistical analyses were performed in R (v. 3.0.0, R Foundation for Statistical Computing, 2008, Vienna, Austria; Packages 'stats', 'nnet', 'MASS' and 'car'), and Figures were drawn in Statistica for Windows (v. 11; Statsoft, 2003, Oklahoma, USA) or MS Excel (installed under Windows 2007). The data distribution and residual deviance of the generalised linear models were always verified so that the model assumptions were not violated. Body mass did not meet the assumptions of a t-test and I therefore employed a non-parametric approach (Mann-Whitney U-test) to compare male and female masses. In all cases initial body mass was a significant predictor of sex, WLR, BWC and time to death. The regression equation for the relation between body mass and sex, WLR, BWC and time to death were shown. Initial body mass was included into analyses as an explanatory covariate and corrected least-square means were plotted unless otherwise specified. Overlap in 95 % confidence limits was used to test for statistical significant homogeneity within and between treatment groups.

4.3 Results

Male moths were significantly lighter than female moths (Mann-Whitney U test, Z = 11.10, $P < 0.001$; Males: 61.06 ± 12.22 mg (std. dev.); females: 119.86 ± 22.17 mg (N = 83 per sex). Male and female body mass was affected by immature stage developmental temperature (Table 4.1) and I therefore included mass as a covariate in subsequent analyses to adjust for size differences between the sexes among acclimation groups. There was no significant interaction effect between acclimation temperature and adult mass on the $BWC_{INITIAL,}$ expressed as a fraction of initial body mass ($\chi^2 = 1.41$, $P = 0.50$). There was a significant positive correlation between $BWC_{INITIAL}$ and body mass (Figure 4.1A, $r^2 = 0.91$, $P < 0.001$) and the interaction effect of developmental

acclimation and sex significantly influenced initial body masses so that female moths were lighter after higher developmental temperature rearing (Figure 4.1B; $\chi^2 = 5.78$, $P = 0.016$).

After correcting for the covariate effect of adult start mass, the results showed support for significant developmental plasticity of adult WLR, time to death and BWC_{CRIT} (Table 4.1). A cooler developmental acclimation temperature (20.0 °C) resulted in a higher WLR and shorter time to death (Figure 4.2A, B). There was a significant positive correlation between WLR and body mass (Figure 4.2C, $r^2 = 0.62$, $P < 0.001$) and a significant negative correlation between time to death and body mass (Figure 4.2D, $r^2 = 0.41$, $P = 0.008$). The linear relationship between WLR (mg/h) and body mass (mg) can be described by the equation: WLR = 0.024 x – 0.120, $r^2 = 0.68$, $P < 0.001$ for 20.0 °C; WLR = 0.026 x – 0.715, $r^2 = 0.54$, $P < 0.001$ for 25.0 °C; WLR = 0.027 x – 0.821, $r^2 = 0.70$, $P < 0.001$ for 30.0 °C where x is initial body mass (mg). The relationship between time to death (h) and body mass (mg) can be represented as a linear fit where time = 0.035 x + 17.918, $r^2 = 0.09$, $P = 0.022$ for 20.0 °C; time = 36.462 – 0.088 x, $r^2 = 0.13$, $P = 0.005$ for 25.0 °C; time = 37.250 – 0.137 x, $r^2 = 0.18$, $P = 0.001$ for 30.0 °C where x is initial body mass (mg).

A warm acclimation temperature (30.0 °C) resulted in a lower $BWC_{INITIAL}$ and higher BWC_{CRIT} in comparison to the other two acclimation treatments (Figure 4.3A, B). The positive correlations between the $BWC_{INITIAL}$ ($r^2 = 0.91$, $P < 0.001$) and BWC_{CRIT} ($r^2 = 0.59$, $P < 0.001$) and body mass were also significant (Figure 4.3C, D). The relationship between $BWC_{INITIAL}$ (mg) and initial body mass (mg) is described by $BWC_{INITIAL} = 0.766$ x + 1.460, $r^2 = 0.91$, $P < 0.001$ where x is initial body mass. The relationship between BWC_{CRIT} (mg) and initial body mass (mg) is described by $BWC_{CRIT} = 0.255$ x – 4.399, $r^2 = 0.79$, $P < 0.001$ for 20.0 °C; $BWC_{INITIAL} = 0.256$ x – 3.356, $r^2 = 0.56$, $P < 0.001$ for 25.0 °C; $BWC_{CRIT} = 0.292$ x – 1.897, $r^2 = 0.55$, $P < 0.001$ for 30.0 °C where x is initial body mass.

Table 4.1. Summary of the generalized linear model results determining the effect of developmental acclimation temperature on estimates of body mass (and sex) in adult *Eldana saccharina* moths, adult moth water loss rate (WLR, mg/h), time to death (h) and critical body water content ($BWC_{INITIAL}$, mg). The degrees of freedom (d.f.), chi-square (χ^2) statistic and *P*-values of the model parameters are shown. Units are shown in parentheses after the trait or variable of interest. Significant effects are highlighted in bold.

Trait	Effect	d.f.	χ^2	*P*-value
Body mass (log mg)	Developmental acclimation	1	13.30	**<0.0010**
	Sex	1	563.87	**<0.0001**
	Developmental acclimation x Sex	1	5.78	**0.0160**
WLR (mg/h)	Body mass	1	264.44	**< 0.0010**
	Developmental acclimation	1	11.67	**< 0.0010**
Time to death (h)	Body mass	1	4.44	**0.0350**
	Developmental acclimation	1	9.17	**0.0030**
$BWC_{INITIAL}$ (log mg)	Body mass	1	2548.90	**< 0.0010**
	Developmental acclimation	1	0.10	0.7460
BWC_{CRIT} (log mg)	Body mass	1	270.99	**< 0.0010**
	Developmental acclimation	1	22.54	**< 0.0010**
$BWC_{INITIAL}$ (% of body mass)	Developmental acclimation	1	2.88	0.0890
BWC_{CRIT} (% of body mass)	Developmental acclimation	1	17.49	**< 0.0010**

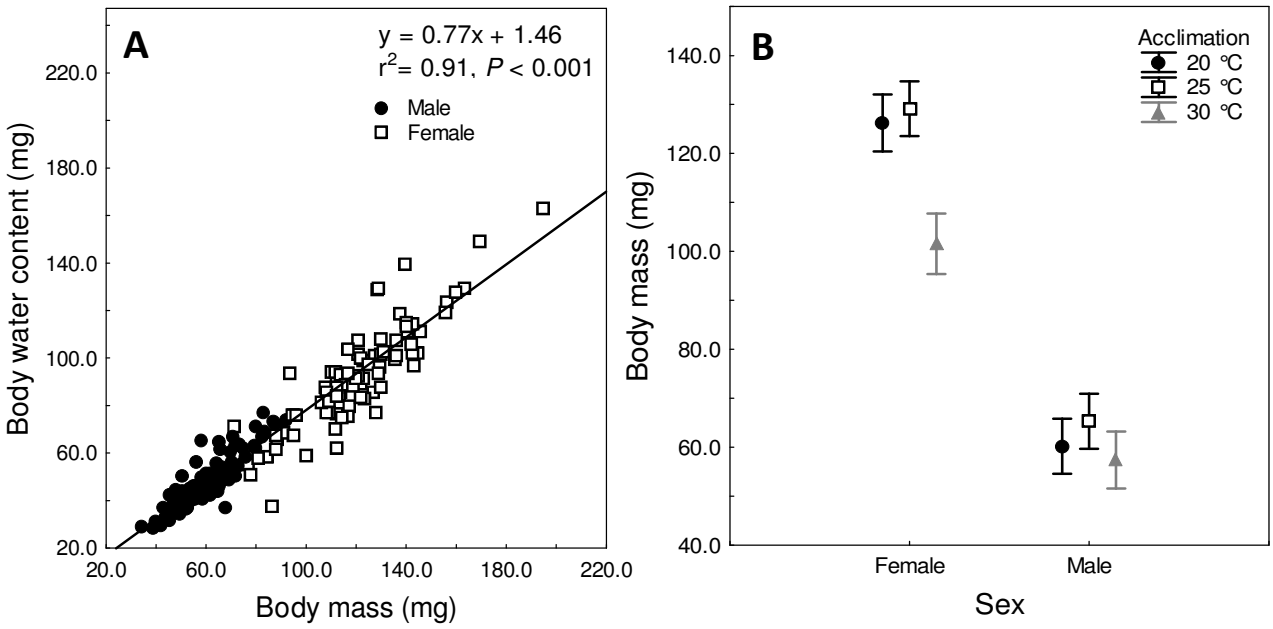

Figure 4.1. (A) Initial body water content ($BWC_{INITIAL}$, mg) for male and female adult *E.*. Acclimation outcomes pooled. (B) Means (± 95 % CI) of body mass as a result of sex and developmental temperature (20.0 °C, 25.0 °C or 30.0 °C) are shown.

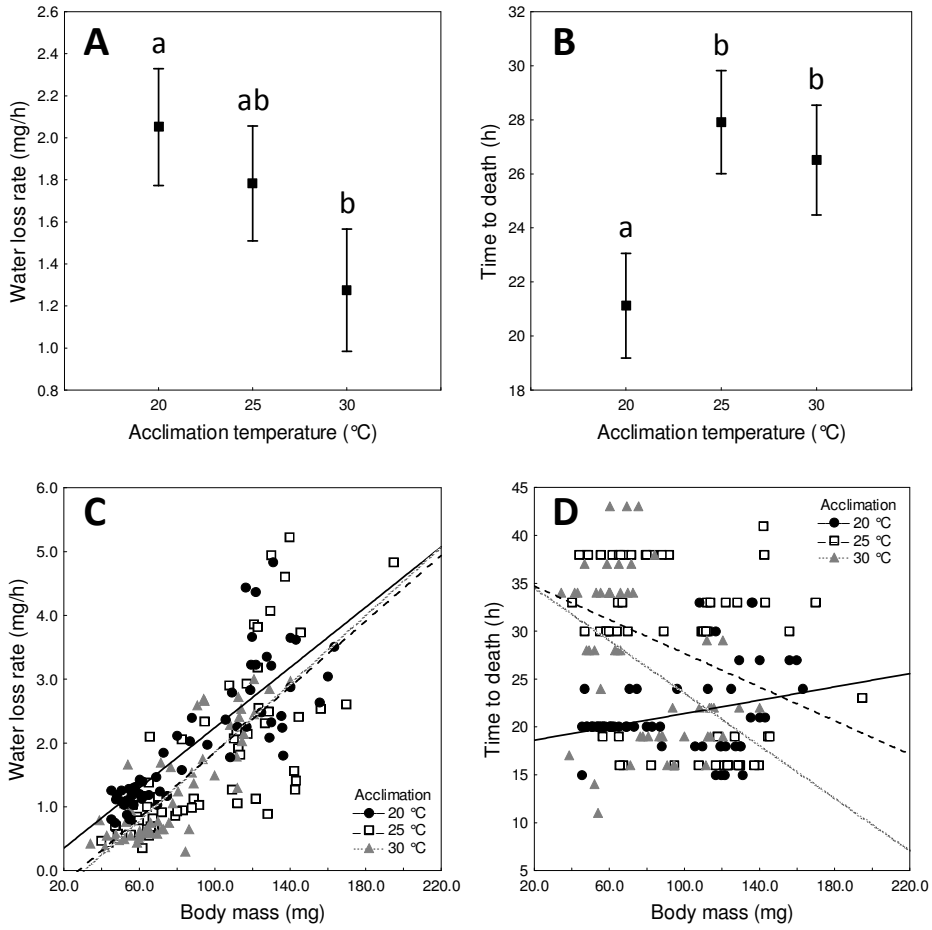

Figure 4.2 (A) Adult moth water loss rate (WLR, mg/h), and (B) time to death (h) at different standard larval acclimation temperatures; and the correlations between water loss rate and body mass (mg) (C) and time to death and body mass (D). Water loss was measured over a 24 h period following developmental acclimation (larva to adult) at 20.0 °C, 25.0 °C or 30.0 °C. The means ± 95 % confidence intervals are shown for the acclimation outcomes and the data are corrected for the continuous predictor: body mass. In the scatterplots different symbols and regression lines represent the three acclimation temperature outcomes.

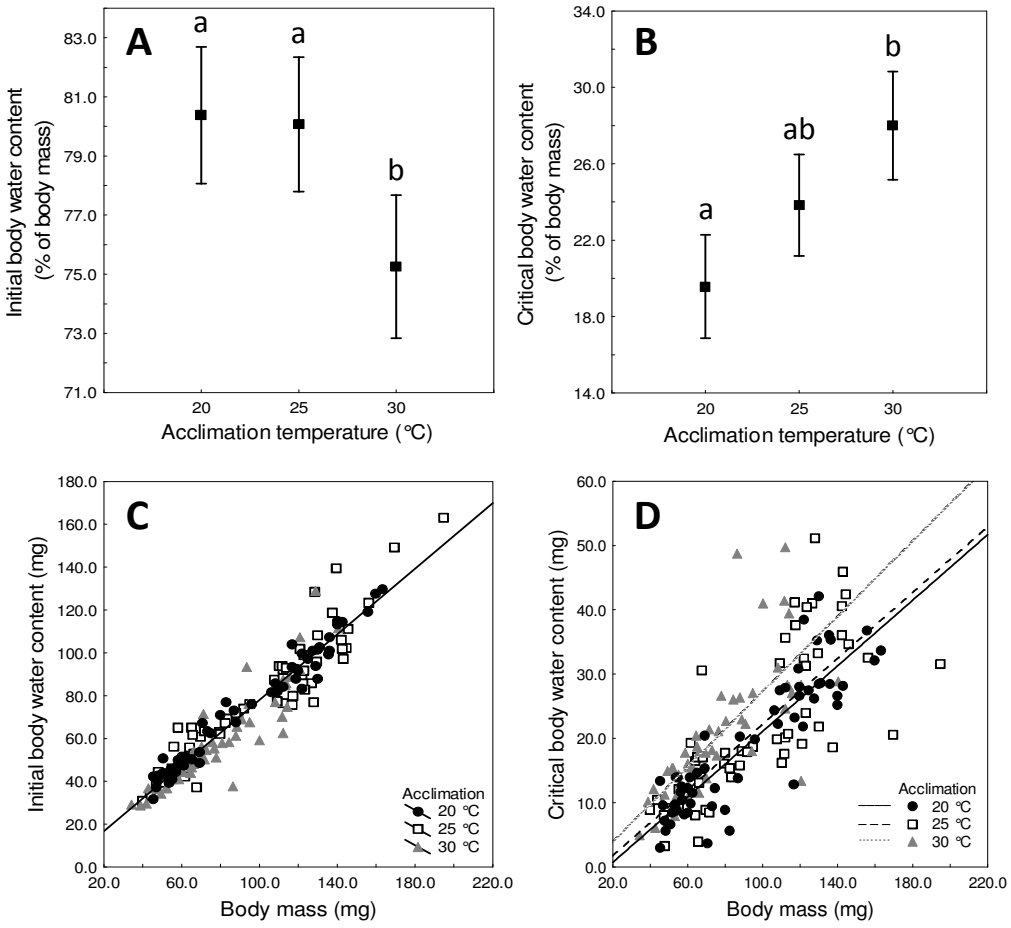

Figure 4.3 (A) Adult moth initial body water content ($BWC_{INITIAL}$, % of body mass) calculated as the difference between dry body mass (mg) and initial body mass (mg), presented as a fraction of initial body mass and (B) critical body water content (BWC_{CRIT}, % of body mass) calculated as the dry body mass minus the body mass at death (mg) presented as a fraction of initial body mass at different larval acclimation temperatures. The means ± 95 % confidence intervals are shown for the acclimation outcomes and the data are corrected for the continuous predictor: body mass. Correlations between body water contents and body mass (C and D) is given with different symbols representing the different acclimation groups.

4.4 Conclusion

The results of this study show pervasive direct effects of rearing temperature experienced by immature stages of the insect on adult water balance related traits in *E. saccharina*. Significant indirect effects were also detected via sex-specific changes in body size and composition (e.g. proportion of body water). These responses of water balance traits suggest a significant role for developmental plasticity of water balance-related traits in the context of thermal variability under field conditions (e.g. at transitions among seasons or between weather fronts) with likely impacts for field activity and population dynamics. Major findings from this study include that plastic responses resulting from developmental temperature acclimation can result in i) increased body water content (or be associated with increased body size, e.g. Chown & Klok, 2003; Kingsolver & Huey, 2006) while the organism is hydrated ($BWC_{INITIAL}$), ii) decreased water loss rate (WLR) via changes in cuticular permeability, respiratory water loss or both (e.g. Bazinet et al., 2010; Terblanche et al., 2010) or iii) increased amount of water that could be lost prior to death. In the latter case, variation in the critical body water content (BWC_{CRIT}) can also influence survival time and enhance insect overall desiccation resistance (Bursell, 1957; Bazinet et al., 2010; Boardman et al., 2013). In addition, I found a positive relationship between WLR and body size (irrespective of sex) and most importantly, support for significantly lower WLRs and smaller body masses (female moths) after high temperature rearing (30.0 °C), even after statistical adjustment was made for size-related variation. Furthermore, significantly lower $BWC_{INITIAL}$ (75.7 ± 1.3 % of initial body mass) were associated with the high temperature rearing group, while significantly higher BWC_{CRIT} were associated with high temperature rearing (28.0 ± 1.5 % of initial body mass) in comparison to low temperature rearing. The results suggest that high temperature rearing results in lower $BWC_{INITIAL}$ while at the same time these rearing conditions led to significantly higher BWC_{CRIT} expressed as a percentage of body mass. This might indicate a better overall ability to withstand desiccating

conditions. Low body water contents at higher acclimation conditions are coupled with low WLRs and greater tolerance to losing high amounts of body water (47.3 % of initial body mass) prior to death.

The $BWC_{INITIAL}$ estimates correspond well with those reported for other Lepidoptera species (range 64 - 84%; Hadley, 1994, Table 2.1, p. 26). Moreover, more than 50 % of $BWC_{INITIAL}$ were lost prior to death across the rearing conditions, which is also similar to e.g. the Tenebrionid beetle *Phrynocolus petrosus* (Zachariassen et al., 1987; see p. 31 in Hadley, 1994). After rearing *E. saccharina* immature stages at 20.0 °C, a smaller fraction (as %) of adult body mass was lost prior to death, irrespective of sex (BWC_{CRIT} = 19.6 ± 0.9 % of body mass) even though high amounts of body water were readily available ($BWC_{INITIAL}$ = 80.4 ± 0.7 % of body mass). Hence, *E. saccharina* have possibly adapted a higher $BWC_{INITIAL}$ in combination with low WLR to cope with, and survive desiccation for longer under high temperature conditions, while low temperatures appear to relax substantial constraints on desiccation resistance and water economy (and see e.g. Terblanche & Kleynhans, 2009). These changes in physiological traits are not associated simply with changes in body size (although clearly males and females responded very differently to similar temperature effects, depending on exact developmental conditions), and considering the way the statistical analyses were run by correcting for the significant covariate effect of body mass. Male moth body size was hardly impacted by rearing temperature, while warm-reared females were smaller than intermediate or cold-reared females (Figure 4.1B). This response is, however, not uncommon since many studies have shown similar sex-dependent effects of temperature on growth rate or final body size (e.g. Chown & Klok, 2003; Blanckenhorn et al., 2006; but see also Fischer & Fiedler, 2000; Ferrer et al., 2014) and is related to the 'temperature size rule' such that hotter conditions typically result in smaller body size (reviewed in e.g. Kingsolver & Huey, 2008).

Cold rearing temperatures led to shorter time to death of both sexes (i.e. higher mortality at a given time), high WLRs and low BWC_{CRIT} (low amounts of water as % of body mass survived), even though high amounts of water (as a % of body mass) were available. Thus, results from the present study can be interpreted as providing support for the view that, at least from a water balance perspective, *E. saccharina* are less capable of adjusting further to higher rearing temperatures (i.e. above their optimal rearing temperature, 25.0 - 27.0 °C). By contrast, lower temperatures result in fundamentally different traits, and even influence the scaling of these traits with body size. Consequently, it seems likely that developmental (phenotypic) plasticity probably plays a significant role in trait variation between populations of *E. saccharina* experiencing different climate conditions (see also Ayrinhac et al., 2004; Hoffmann et al., 2005; Terblanche et al., 2006). Comparisons among populations will therefore need to account for plastic effects in order to compare water balance traits, as is also the case for traits of thermal stress resistance in *E. saccharina* (e.g. Kleynhans et al., 2014c).

In conclusion, plastic responses of water balance-related traits of *E. saccharina* adults resulted in an increased $BWC_{INITIAL}$, decreased WLR and increased amount of body water that could be lost prior to death under warmer rearing conditions. This enhanced *E. saccharina* desiccation resistance when tested as adults (see also Bazinet et al., 2010; Terblanche et al., 2010). Thus, developmental acclimation within a single generation to a higher temperature resulted in a positive developmental plasticity of water balance-related traits measured in adult *E. saccharina*. After colder developmental acclimation an increased WLR resulted in a shorter time to death, while an increased adult moth survival time might result from higher $BWC_{INITIAL}$ upon death after warm developmental acclimation. These results are significant for understanding the responses of insects to rearing temperature under field and laboratory conditions, and suggest that variation in temperature during development significantly alters the ability of adults to withstand desiccation.

Further work on the mechanisms (e.g. changes in cuticular lipid composition, respiratory water loss adjustments) underlying these responses would be useful.

4.5 References

Addo-Bediako, A., Chown, S. L. & Gaston, K. J. (2001) Revisiting water loss in insects: a large scale view. Journal of Insect Physiology 47: 1377–1388.

Addo-Bediako, A., Chown, S. L. & Gaston, K. J. (2002) Metabolic cold adaptation in insects: a large-scale perspective. Functional Ecology 16: 332–338.

Assefa, Y., Conlong, D. E. & Mitchell, A. (2005) Mitochondrial DNA variation among populations of sugarcane borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Proceedings of the South African Sugar Technologists' Association 79: 382–385.

Ayrinhac, A., Debat, V., Gibert, P., Kister, A–G., Legout, H., Moreteau, B., Vergilino, R. & David, J. R. (2004) Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. Functional Ecology 18: 700–706.

Bazinet, A. L., Marshall, K. E., MacMillan, H. A., Williams, C. M. & Sinclair, B. J. (2010) Rapid changes in desiccation resistance in *Drosophila melanogaster* are facilitated by changes in cuticular permeability. Journal of Insect Physiology 56: 2006–2012.

Bedick, J. C., Hoback, W. W. & Albrecht, M. C. (2006) High water-loss rates and rapid dehydration in the burying beetle, *Nicrophorus marginatus*. Physiological Entomology 31: 23–29.

Benoit, J. B., Patrick, K. R., Desai, K., Hardesty, J. J., Krouse, T. B. & Denlinger, D. L. (2010) Repeated bouts of dehydration deplete nutrient reserves and reduce egg production in the mosquito *Culex pipiens*. Journal of Experimental Biology 213: 2763–2769.

Blanchenhorn, W. U., Stilwell, R. C., Young, K. A., Foxm C. W. & Ashton, K. G. (2006) When Rensch meets Bergmann: does size dimorphism change systematically with latitude? Evolution 60: 2004–2011.

Boardman, L. Sørensen, J. G. & Terblanche, J. S. (2013) Physiological responses to fluctuating thermal and hydration regimes in the chill susceptible insect, *Thaumatotibia leucotreta*. Journal of Insect Physiology 59: 781–794.

Bursell, E. (1957) Spiracular control of water loss in the tsetse fly. Proceedings of the Royal Entomological Society of London A 32: 21–29.

Chown, S. L. & Klok, C. J. (2003) Water-balance characteristics respond to changes in body size in subantarctic weevils. Physiological and Biochemical Zoology 76: 634–643.

Chown, S. L., Sørensen, J. G. & Terblanche, J. S. (2011) Water loss in insects: an environmental change perspective. Journal of Insect Physiology 57: 1070–1084.

Conlong, D. E. (1989) *Technique development for mass rearing*. Proceedings of the first insect rearing workshop held during the seventh national congress of the Entomological Society of Southern Africa. pp. 10–14.

Cooper, B. S., Czarnoleski, M. & Angilletta Jr., M. J. (2010) Acclimation of thermal physiology in natural populations of *Drosophila melanogaster*: a test of an optimality model. Journal of Evolutionary Biology 23: 2346–2355.

Deere, J. A. & Chown, S. L. (2006) Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. The American Naturalist 168: 630–644.

Dell, A. I., Pawar, S. & Sabage, V. M. (2011) Systematic variation in the temperature dependence of physiological and ecological traits. Proceedings of the National Academy of Sciences of the USA 108: 10591–10596.

Dick, J. (1945) Some data on the biology of the sugarcane borer (*Eldana saccharina Wlk*). Proceedings of the South African Sugar Technologists' Association 19: 75–79.

Dillon, M. E., Wang, G. & Huey, R. B. (2010) Global metabolic impacts of recent climate warming. Nature 467: 704–707.

Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R. & Mearns, L. O. (2000) Climate extremes: observations, modelling, and impacts. Science 289: 2068–2074.

Fallis, L. C., Fanara, J. J. & Morgan, T. J. (2014) Developmental thermal plasticity among *Drosophila melanogaster* populations. Journal of Evolutionary Biology 27: 557–564.

Ferrer, A., Mazzi, D. & Dorn, S. (2014) Stay cool, travel far: cold-acclimated oriental fruit moth females have enhanced flight performance but lay fewer eggs. Entomologia Experimentalis et Applicata 151: 2014, 11–18.

Fisher, K. & Fiedler, K. (2000) Sex-related differences in reaction norms in the butterfly *Lycaena tityrus* (Lepidoptera: Lycaenidae). Oikos 90: 372–380.

Fortain, B., Comberlin, P., Louvet, S., Oettli, P. & Valimba, P. (2010) Climate adjustments over Africa accompanying the monsoon onset. J. Climate 23: 2047–2064.

Gaston, K. J. & Chown, S. L. (2013) Macroecological patterns in insect body size. In: Body size: Linking pattern and process across space, time and taxonomic group (Smith, F. A. & Lyons, S. K. eds.), pp. 13–61. University of Chicago Press, Chicago.

Gibbs, A. G., Perkins, M. C. & Markow, T. A. (2003) No place to hide: microclimates of Sonoran Desert *Drosophila*. J. Therm. Biol. 28: 353–362.

Gillespie, D. (1993) Development of mass-rearing methods for the sugarcane borer *Eldana saccharina* (Lepidoptera: Pyralidae) ii: Diet gelling agents. Proceedings of the South African Sugar Technologists' Association 67: 127–129.

Girling, D. J. (1978) The distribution and biology of *Eldana saccharina* Walker (Lepidoptera Pyralidae) and its relationship to other stem-borers in Uganda. Bull. Ent. Res. 68: 471–488.

Gray, E. M. & Bradley, T. J. (2005) Evidence from mosquitoes suggests that cyclic gas exchange and discontinuous gas exchange are two manifestations of a single respiratory pattern. Journal of Experimental Biology 209: 1603–1611.

Hadley, N. F. (1994) Water relations of terrestrial arthropods. Academic Press, San Diego.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones P. G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. **25**: 1965–1978.

Hijmans, R. J., Cameron, S.E., Parra, J. L., Jones, P. G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965–1978.

Hoffmann, A. A. (1995) Acclimation: increasing survival at a cost. Trends in Ecology and Evolution 10: 1–2.

Hoffmann, A. A., Hallas, R. J., Dean, J. A. & Schiffer, M. (2003) Low potential for climatic stress adaptation in a rainforest *Drosophila* species. Science, 301: 100–102.

Hoffmann, A. A. & Harshman, L. G. (1999) Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population and intrapopulation levels. Heredity 83: 637–643.

Hoffmann, A. A., Shirriffs, J. & Scott, M. (2005) Relative importance of plastic *vs* genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. Functional Ecology 19: 222–227.

Huey, R. B. & Berrigan, D. (1996) Testing evolutionary hypotheses of acclimation. In: Animals and Temperature. Phenotypic and Evolutionary Adaptation (Johnston, I. A. & Bennett, A. F. eds.), pp. 205–237. Cambridge University Press, Cambridge.

Huey, R. B., Berrigan, D., Gilchrist, G. W. & Herron, J. C. (1999) Testing the adaptive significance of acclimation: a strong inference approach. American Zoology 39: 323–336.

Kearney, M. (2012) Metabolic theory, life history and the distribution of a terrestrial ectotherm. Functional Ecology 26: 167–179.

Kellermann, V., van Heerwaarden, V., Sgrò, C. M. & Hoffmann, A. A. (2009) Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. Science 325: 1244–1246.

Kingsolver, J. G. & Huey, R. B. (2008) Size, temperature, and fitness: three rules. Evolutionary Ecology Research 10: 251–268.

Kleynhans, E., Clusella-Trullas, S. & Terblanche, J. S. (2014a). Impacts of environmental variability on desiccation rate, plastic responses and population dynamics of *Glossina pallidipes*. Journal of Evolutionary Biology 27, 337–348.

Kleynhans, E., Conlong, D. E. & Terblanche, J. S. (2014b) Host plant-related variation in thermal tolerance of *Eldana saccharina* (Lepidoptera: Pyralidae). Entomologia Experimentalis et Applicata 150: 113–122.

Kleynhans, E., Mitchell, K. A., Conlong, D. E. & Terblanche, J. S. (2014c) Evolved variation in cold tolerance among populations of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa. Journal of Evolutionary Biology 27: 1149–1159.

Kleynhans, E. & Terblanche, J. S. (2011) Complex interactions between temperature and relative humidity on water balance of adult tsetse (Glossinidae, Diptera): implications for climate change. Frontiers in Physiology 2, doi: 10.3389/fphys.2011.00074.

Leinaas, H. P., Slabber, S. & Chown, S. L. (2009) Effects of thermal acclimation on water loss rate and tolerance in the collembolan *Pogonognathellus flavescens*. Physiological Entomology 34: 325–332.

Marron, M. T., Markow, T. A., Kain, K. J. & Gibbs, A. G. (2003) Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. Journal of Insect Physiology 49: 261–270.

Overgaard, J., Kearney, M. R. & Hoffmann, A. A. (2014) Sensitivity to thermal extremes in Australian *Drosophila* implies similar impacts of climate change on the distribution of widespread and tropical species. Glob. Change Biol. doi: 10.1111/gcb.12521

Parkash, R., Tyagi, P. K., Sharma, I. & Rajpurohit, S. (2005) Adaptations to environmental stress in altitudinal populations of two *Drosophila* species. Physiological Entomology 30: 353–361.

Piersma, T. & van Gils, J. A. (2011) The flexible phenotype: A body–centred integration of ecology, physiology and behaviour. Oxford University Press, UK.

R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org.

StatSoft, I. (2003) STATISTICA (data analysis software system), version 11. http://www.statsoft.com.

Terblanche, J. S. & Chown, S. L. (2006) The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae). Journal of Experimental Biology 209: 1064–1073.

Terblanche, J. S., Clusella-Trullas, S. & Chown, S. L. (2010) Phenotypic plasticity of gas exchange pattern and water loss in *Scarabaeus spretus* (Coleoptera: Scarabaeidae): deconstructing the basis for metabolic rate variation. Journal of Experimental Biology 213: 2940–2949.

Terblanche, J. S. & Kleynhans, E. (2009) Phenotypic plasticity of desiccation resistance in *Glossina* puparia: are there ecotype constraints on acclimation responses? Journal of Evolutionary Biology 22: 1636–1648.

Terblanche, J. S., Klok, J. C., Krafsur, E. S. & Chown, S. L. (2006) Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): Implications for distribution modelling. The American Journal of Tropical Medicine and Hygiene 74: 2006, 786–794.

Terblanche, J. S., Sinclair, B. J., Klok, C. J., McFarlane, M. L. & Chown, S. L. (2005) The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalcoptera* (Coleoptera: Chrysomelidae). Journal of Insect Physiology 51: 1013–1023.

Way, M. J. (1994) A preliminary assessment of the effects of different constant temperatures on the reproduction of *Eldana saccharina* (Lepidoptera: Pyralidae). Proceedings of the South African Sugar Technologists' Association 68: 16-18.

Way, M. J. (1995) Developmental biology of the immature stages of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Proceedings of the South African Sugar Technologists' Association 69: 83–86.

Winston, P. W. & Bates, D. H. (1960) Saturated solutions for the control of humidity in biological research. Ecology 41: 232–237.

Woods, H. A. & Smith, J. N. (2010) Universal model for water cost of gas exchange by animals and plants. Proceedings of the National Academy of Sciences of the USA 107: 8469–8474.

Zachariassen, K. E., Kamau, J. M. Z. & Maloiy, G. M. O. (1987) Water balance and osmotic regulation in the East African tenebrionid beetle *Phrynocolus petrosus*. Comparative Biochemistry and Physiology 86: 79–83.

Chapter 5

Local climate affects temporal population performance of *Eldana saccharina* Walker (Lepidoptera: Pyralidae)

5.1 Introduction

Lepidopteran borers are food crop pests of economic concern in sub-Saharan Africa (Davis & Pedigo, 1990; Cardwell et al., 1997; Kfir, 1998). The African sugarcane stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae), poses a significant threat to commercial, emerging and small-scale sugarcane growers in South Africa, Zimbabwe, Uganda, Ethiopia, and west African countries (Girling, 1972; Conlong, 2001; Assefa et al., 2010). Larval boring (i.e. feeding) causes direct and indirect revenue losses through stalk damage and secondary stalk infections respectively, both affecting sugarcane yield (Butterfield, 2002; Goebel & Way, 2006). Control strategies for *E. saccharina* includes cultural practices, chemical- and biological intervention (e.g Webster et al., 2006). Most recent developments include integrated pest management (IPM) programs that are followed by commercial sugarcane growers (Webster et al., 2006) and more so, intensive research and the implementation of area-wide IPM programs at the South African Sugarcane Research Institute (SASRI) (Conlong & Rutherford, 2009; Webster et al., 2009).

In South Africa, the first pest outbreak in sugarcane occurred in 1939 on two-year-old sugarcane (cultivar: P.O.J. 2725) planted in the warm Umfolozi flats of KwaZulu-Natal (Dick, 1945; Carnegie, 1974; Atkinson, 1980). Previous studies concluded that low winter temperatures would limit the distribution of this stalk borer through its impact on adult reproductive performance, larval development and survival (Dick, 1945; Atkinson, 1980; Way, 1994). Female mating success was highest at 25.0 °C and mating did not happen when temperatures were below 18.0 °C at sunset (Dick, 1945). At low temperatures (11.0 °C for 14 days) larvae were completely inactive and did not feed, but, when returned to 20.0 – 25.0 °C revived and developed further (Dick, 1945). Furthermore, larval development slowed down

during hibernation when average temperatures were around 17.0 °C during the winter season and otherwise developed normally throughout the year (Dick, 1945). Kleynhans et al. (2014a) showed that freezing is lethal for *E. saccharina* pupae and this happened at an average temperature of -17.0 ± 1.8 s.e. °C and the lower developmental threshold for *E. saccharina* pupae were 11.6 °C according to Way (1995). Unpublished results from some preliminary work on the freeze tolerance strategy of *E. saccharina* larvae showed that freezing (which happened at -7.0 ± 0.3 °C) was lethal. Cold temperature tolerance assays revealed that *E. saccharina* larvae lost coordinated muscle function at 7.6 ± 0.1 °C. Since the first *E. saccharina* outbreak in the Umfolozi flats, *E. saccharina* has spread rapidly into novel areas formerly thought to be too cold for the completion of the larval life stage (Webster et al., 2006; Assefa et al., 2008). As such, the current known geographic distribution of *E. saccharina* stretches across distinct climates (e.g. Conlong, 2001; Kleynhans et al., 2014b) beyond the coastal sugar belt to inhabit the warm sub-tropical summer climates in Thohoyandou in Limpopo (northern limit), cool winters in the Mkambati Nature Reserve in the Eastern Cape (southern limit) and cold winter sites with occasional frost at Boskop dam in the North-West province (western limit) (Conlong, 2001; Assefa et al., 2008).

Across the geographic distribution of *E. saccharina*, populations at different climatic locations could represent different biotypes that differ ecologically (see Conlong, 2001). These differences might result in phenotypic variability (Maes, 1998) and genetic variation between populations (e.g. King et al., 2002; Chown et al., 2007; Kleynhans et al., 2014b). Webster et al. (2006) concluded that frequent frost occurrences in the northern Midlands possibly lead to *E. saccharina* absence (see Table 1 in Webster et al., 2006; 2009), however, *E. saccharina* are spreading and possibly adapting to the cooler climates of the Midlands of

KwaZulu-Natal. Indeed, there is marked phylogenetic differentiation between *E. saccharina* populations along geographical lines in broader Africa (Lange et al., 2004; Assefa et al., 2006), and furthermore, there is evidence for phenotypic variation of climate stress resistance traits of *E. saccharina* within South Africa likely through the effects of local scale climate variation (Kleynhans et al., 2014b) and perhaps also host plant differences at the finer scale (Kleynhans et al., 2014a).

Here I aim to develop a better understanding of the effect of local climate on population phenology, fitness and abundance of *E. saccharina* in South Africa. The null-hypothesis that local climate and furthermore over-wintering stage did not have a significant effect on population phenology, fitness and abundance is tested. A mechanistic (process-based) population model is applied, following the methods described in Barton & Terblanche (2014), to predict population performance at two geographic locations currently occupied by *E. saccharina*: a warm site and a cold site. The two sites were chosen to represent the extremes of their current thermal range, at least within South Africa. More specifically, life-stage specific developmental rates and critical temperature thresholds were integrated with hourly temperature data to predict how seasonal fluctuations in thermal conditions at the two study sites may affect their phenology, thermal stress potentially experienced, survival, population turnover, relative abundance and fecundity of *E. saccharina*. In addition to modelling the outcomes at the two climatically-distinct geographic locations, two separate freeze-intolerant over-wintering life stage scenarios (larvae *vs.* pupae) were considered. This is due to in-field scouting data revealing the presence of both stages in the sugarcane at the two study sites during mid-winter, as well as previous literature which concluded that larvae become inactive at cold conditions and cease to feed, however, when returned to warmer

conditions revive and develop further (Dick, 1945). This suggests that larval development can be slowed down, but continues during the winter season (i.e. are capable of surviving overwinter).

5.2 Materials and methods

A mechanism-based population model (see Barton & Terblanche, 2014) was applied to high resolution time-series climate data for two point locations in South Africa, where *E. saccharina* is currently found in sugarcane: a relatively warm site near Umfolozi (28°50′17″S, 31°53′44″E, 15 m.a.s.l.) and a colder site near Eston (29°52′00″S, 30°31′00″E, 785 m.a.s.l) in the province of KwaZulu-Natal. For each site, hourly temperature fluctuations were interpolated from measurements of daily average minimum and maximum temperatures with a sinusoidal wave according to the equation in Campbell & Norman (1998). Average daily temperature records were obtained from the SASRI Weather Data Acquisition and Processing System (WeatherWeb).

At each hourly time step, the model calculated the number of degree-day (DD) units obtained according to the ambient temperature, above the lower temperature developmental threshold for the particular life stage (Way, 1995). Physiological data of each life-history stage were called into the model while it was stepping through the temperature data. Thus, as the model progressed through the simulation period, DD units were allowed to accumulate until the threshold DD (for that particular stage) was reached, at which point the model transitioned into the next life stage. Once the adult stage was reached (see Feng et al., 2010), the model remained as an adult for 14 days, after which it transitioned back to an egg, the accumulated

DD total was reset to zero, and an additional generation was tallied (Barton & Terblanche, 2014).

It was assumed that body temperatures (T_b) for *E. saccharina* life stages were equal to ambient temperature in the sugarcane field for the purpose of modelling the population responses throughout the season, i.e. the thermal effects of radiation, metabolism and evaporation on body temperatures of this species were assumed to be negligible (see Watt, 1968; Kingsolver & Moffat, 1982). This assumption is based on microclimate recordings made in a sugarcane field at three different locations relative to the sugarcane stalk: 1) in the leaf sheaths where eggs are most probably deposited and adult moths can be found (Atkinson, 1979), 2) at the base of the stalk (ground level, where pupae have been seen) and 3) inside the stalk where larvae feed. Furthermore, developing life-stages are seldom exposed to direct solar radiation. During the mid-winter and –summer months, temperatures were recorded for 21 consecutive days using calibrated thermochron iButton data loggers (8-bit Model DS1921; iButton, Dallas, TX, USA; 0.5 °C accuracy) set to record at 30-minute intervals. There were no significant difference between the temperatures at the three locations in the sugarcane stalk (N = 1924 data points per stalk location, GLZ: $\chi^2 = 4.71$, d.f. = 2, $P = 0.10$).

Thermal constants and lower developmental thresholds for the life-history stages were obtained from work published by Way (1995). A Briere model (i.e. quadratic temperature-dependent equation) was used to estimate a theoretical optimal temperature for each life stage (Briere et al., 1999). The input parameters for the model are given in Table 5.1.

During the larval phase, *E. saccharina* transitions through 5 – 8 instars, depending on their diet and gender (Waiyaki, 1968; Girling, 1978; Atkinson, 1980). A seven-instar larval life

stage was modelled, which corresponds to findings by Atkinson (1980), and the age-related head capsule size studies completed by Way (1995). The temperature-dependent rate of egg-production was also obtained from Way's (1995) work and corresponded to 205, 417, 432 and 183 eggs at 15.0, 20.0, 25.0 and 30.0 °C, respectively.

Table 5.1. Thermal constant (K) and lower developmental threshold (LDT) estimates for the different stages, obtained from work done by Way (1995) and estimates of the theoretical optimal temperature (T_{opt}) for development of each life stage (following Briere et al., 1999).

Stage	Name	K	LDT	T_{opt}
1	Egg	119.0	4.5	28.0
2	Larva 1	80.0	11.2	29.0
3	Larva 2	70.0	9.7	29.0
4	Larva 3	69.0	9.0	29.0
5	Larva 4	74.0	9.5	29.0
6	Larva 5	86.0	11.0	29.0
7	Larva 6	129.0	11.0	29.0
8	Larva 7	116.0	12.3	30.0
9	Pupae	160.0	11.6	29.0
10	Adult	200.0	11.0	28.0

For each geographic location, two model simulations were run to explore the outcomes of over-wintering as either a pupa, or larva. The model was set to commence on 2013-07-01 with accumulated DD units set to 624.0 °D and 160.3 °D for the larva and pupa over-wintering scenarios, respectively. For each modelling scenario, estimates of phenology and fitness were subsequently tracked throughout the 365 day simulation period. Model output parameters included: the number of generations per year (and showing the life-stage transitions throughout the season), number of stress hours encountered through each life-stage and for the season, relative fitness of adult moths, relative adult abundance and female fecundity that were compared between sites and over-wintering life stage. Briefly, for each hour, DD were calculated where temperatures did not exceed 35.0 °C or dropped below the lower developmental threshold (LDT). Stress-hours were calculated as the total number of hours during which body temperature (T_b) dropped below an arbitrary stress-inducing cold temperature set to 2.0 °C below the LDT (cold stress) or rose above 35.0 °C (heat stress). The thermal safety margin (T_{opt} - T_b) were calculated for each hour. Relative population fitness was calculated for emerging adult moths, based on the whole developmental period's thermal safety margin (TSM), where relative fitness = 1/TSM, so adults that had developmental periods closest to the optimum were assumed to be the fittest (e.g. Huey & Stevenson, 1979). The relative abundance was calculated as the total number of adult female emergences over the previous 14 day period, assuming female moths survive for two weeks in total, multiplied by the relative fitness at that hour. Female fecundity predictions were estimated based on a linear model of the relationship between temperature and egg-production (see above for data). The number of reproducing two-day old adult moths on each day were calculated and multiplied by the number of eggs that could be produced during that hour, depending on the T_b, and the temperature-dependent function for egg production.

Model predictions were validated against weekly-obtained scout records (expressed as a % larvae [all instars included] scouted per 100 stalks) obtained from 29 sugarcane fields (> 3ha average, 7 – 31 month old sugarcane, 16 different cultivars) in and around the approximate model site. Predictions of life-stage transition and stress (cold and heat) were used at the Eston site with a larval over-wintering stage scenario, because scouted larval numbers returned high in-field infection during the initial winter months of this particular season. Scout records were obtained from the South Africa Sugarcane Research Institutes' extension specialist in the Midlands South region of KwaZulu-Natal (in the area of 29°55′12.46″S, 30°38′56.06″E and 29°55′03.18″S, 30°39′07.68″E). Surveys for *E. saccharina* larvae are done by Pest and Disease survey teams that pluck 100 stalks at random from a < 10 ha sugarcane field. The stalks are sliced in half, lengthwise, and the number of larvae found are expressed as a percentage infestation, i.e. indication of larval relative abundance. The field age and cultivar are recorded. The cultivars included in the scout records obtained from the extension specialist were high risk varieties: N16, N28, N31, N35, N36, N37, N40, N45, N47, N48, N49, N50, N52 and low risk varieties: N12, N39, N41. The sugarcane ages where scouting were done ranged between 7 – 31 months.

All statistical analyses were performed in R (v. 3.1, R Foundation for Statistical Computing, Vienna, Austria; Packages 'MASS' and 'car'). A comparison of ambient temperature was made between the geographic locations using the Wilcoxon-Mann-Whitney rank sum test as data were not normally distributed. The effect of geographic location and over-wintering life stage on the number of generations, cold- and heat-stress hours were compared, with Poisson distribution of errors and log link function, while fecundity and relative fitness were compared, with Gaussian distribution of errors and identity link function, in generalised

linear models. The models were corrected for over-dispersion by square root transformation of count data (quasi- family distribution of errors did not stabilize the variance in the data) and log transformation of continuous data. The models were checked for over-dispersion by inspection of residual deviance and degrees of freedom (following Crawley, 2007).

5.3 Results

Population responses were modelled at two geographic sites from mechanistic principles, initiated from a larval or pupal over-wintering life stage. Statistical analyses of the calculated ambient hourly temperature verified that the Umfolozi site (mean ± SD: 21.5 ± 3.9 °C) was significantly warmer than at the Eston site (18.3 ± 3.9 °C) ($W = 22375584$, $P < 0.0001$). Variation in ambient temperatures between the two sites resulted in significant differences in predicted fitness traits of *E. saccharina*.

As expected, the number of generations completed for *E. saccharina* across the season was higher at the warm site than at the cold site (Figure 5.1A, B). The interaction effect between the site and over-wintering life stage on the number of generations completed was significant (Table 5.2), reflected by an additional generation following pupal over-wintering at the cold site (Figure 5.1A, B). At the warm site, while overwintering stage had an impact on the timing of life-history transitions, three generations could be completed under both over-wintering model scenarios. In contrast, at the cold site, generation number depended on the over-wintering life stage: when the winter months were spent as pupae, two generations could be completed in comparison to only one generation if larvae overwintered. In the latter scenario however, where generation numbers of the larval over-wintering stage were tallied, a second generation was very close to completion in the modelled season.

Further information was obtained from the model on the timing of life-history transitions. At the warm site, adult moths were predicted to be present in October, January and March – April (Figure 5.1C, D) irrespective of over-wintering stage. At the cold site adult moth peaks were predicted in January following larval over-wintering (Figure 5.1C) and in November – December, as well as March following pupal over-wintering (Figure 5.1D). Thus, larval over-wintering in the cold site reduced the number of hours during which adults were predicted to be present in the field, probably through an increased amount of thermal stress-hours experienced in the field.

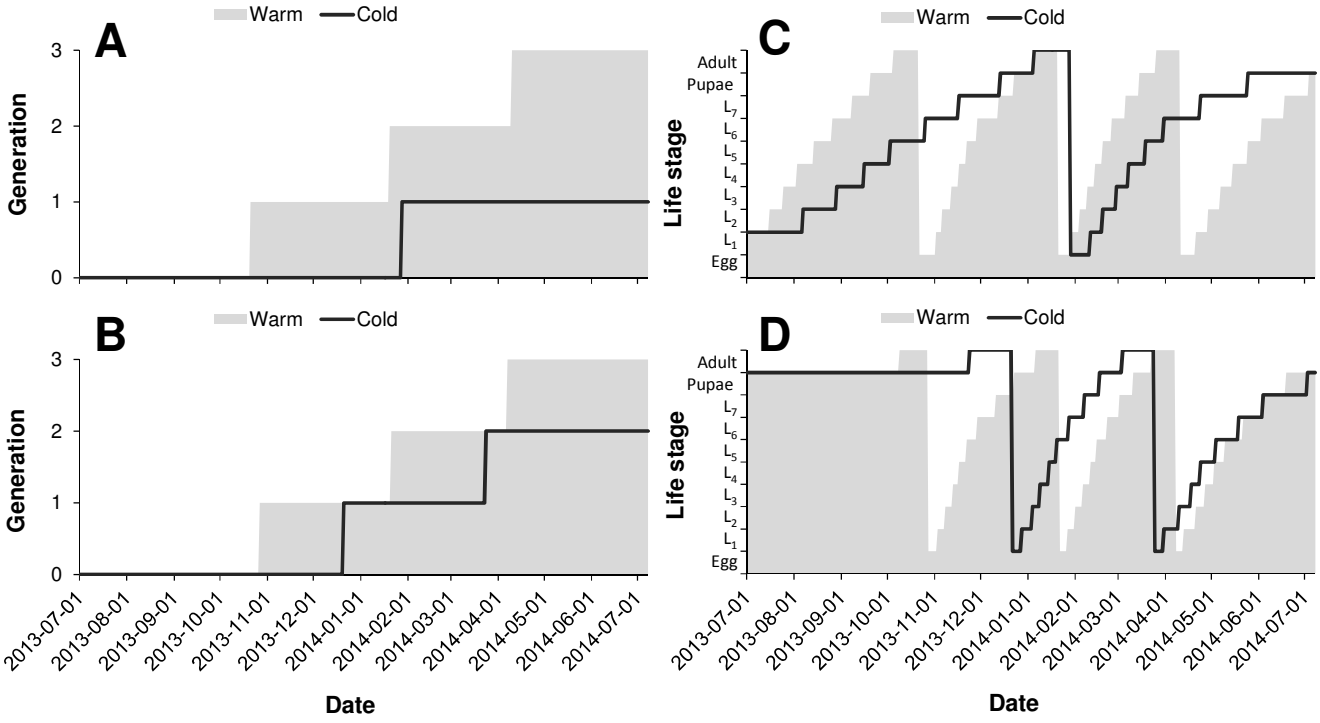

Figure 5.1 The impact of local climate and over-wintering stage on A & B) the number generations completed and C & D) life stage transition predictions for *E. saccharina*. Model predictions were based on a A & C) larval and B & D) pupal over-wintering stage and projected the time steps of life stage transition or generation turn-over over time. "L" on the y axis represents larval instar stage (L_1 = first instar larva).

Table 5.2. Summary results from a generalized linear model results testing the effects of geographic location (Site) and over-wintering life stage (Stage) on the number of generations, cold- and heat-stress hours, relative adult abundance, fecundity (Poisson distribution of errors and log link function) and relative fitness (Guassian distribution of errors and identity link function). Interaction effects are presented with × and the degrees of freedom (d.f.), chi–square (χ2) statistic, corresponding *P*–value are shown. Significant effects are shown in bold.

Model output	Effect	d.f.	χ^2	*P*-value
Number of generations	**Stage**	**1**	**126.59**	**< 0.0001**
	Site	**1**	**2061.07**	**< 0.0001**
	Stage × Site	**1**	**277.48**	**< 0.0001**
Cold-stress (hours)	**Over-winter stage**	**1**	**1184.40**	**< 0.0001**
	Site	**1**	**6501.90**	**< 0.0001**
	Life stage	**9**	**3841.80**	**< 0.0001**
	Over-winter stage × Site	**1**	**1468.90**	**< 0.0001**
	Over-winter stage × Life stage	**9**	**2079.60**	**< 0.0001**
	Site × Life stage	**9**	**2752.00**	**< 0.0001**
	Over-winter stage × Site × Life stage	**9**	**1928.80**	**< 0.0001**
Heat stress (hours)	**Over-winter stage**	**1**	**1065.01**	**< 0.0001**
	Site	**1**	**995.77**	**< 0.0001**
	Life stage	**9**	**356.64**	**< 0.0001**
	Over-winter stage × Site	**1**	**1215.74**	**< 0.0001**

	Over-winter stage × Life stage	**9**	**476.34**	**< 0.0001**
	Site × Life stage	**9**	**315.50**	**< 0.0001**
	Over-winter stage × Site × Life stage	**9**	**682.03**	**< 0.0001**
Heat stress (hours)	**Stage**	**1**	**106.40**	**< 0.0001**
	Site	**1**	**76.35**	**< 0.0001**
	Stage × Site	**1**	**97.62**	**< 0.0001**
Relative adult fitness (1/TSM*)	Stage	1	0.17	0.6819
	Site	**1**	**1659.30**	**< 0.0001**
	Stage × Site	1	0.34	0.5625
Relative adult abundance (adults / day)	Stage	1	0.19	0.6661
	Site	**1**	**231.91**	**< 0.0001**
	Stage × Site	1	0.19	0.6661
Fecundity (eggs / day)	**Stage**	**1**	**5.06**	**0.0246**
	Site	**1**	**46.98**	**< 0.0001**
	Stage × Site	**1**	**7.10**	**< 0.0010**

*TSM = Thermal safety margin

The accumulated number cold and heat stress hours was significantly affected by the interaction effect between over-wintering stage, climate (site) and the different life stages (Table 5.2). More specifically, first and second instar larvae experiences the highest amount of cold stress hours when the larval life stage was over-wintering at a cold site, followed by pupae then the third instar larva. The final larval instar experienced a higher amount of cold stress when pupae over wintered at a cold site. Adult moths experiences the highest amount of heat stress when larvae over wintered at a cold site. In general, the larval life-stage experienced more stress in a cold environment following larval over-wintering in comparison to a warm site and pupal over-wintering (Figure 5.2A, B). In addition, pupae experienced more stress hours at a cold site following larval over-wintering than in a warm site (Figure 5.2A, B). As expected, for pupal over-wintering, *E. saccharina* experienced a higher incidence of heat stress at the warm site in comparison to the cold site, and more cold stress at the cold site in comparison to the warm site (Figure 5.2C, D). Over-wintering larvae were, however, exposed to increased cold and heat stress at the cold site, in comparison to the warm site (Figure 5.2A, B). Cold stress was more frequent at the warm site following pupal over-wintering in comparison to larval over-wintering (Figure 5.2A, C), and both heat and cold stress were more frequent following larval over-wintering, in comparison to pupal over-wintering, at the cold site (Figure 5.2B, D). Relative population fitness and relative adult abundance are direct results of population stress-predictions.

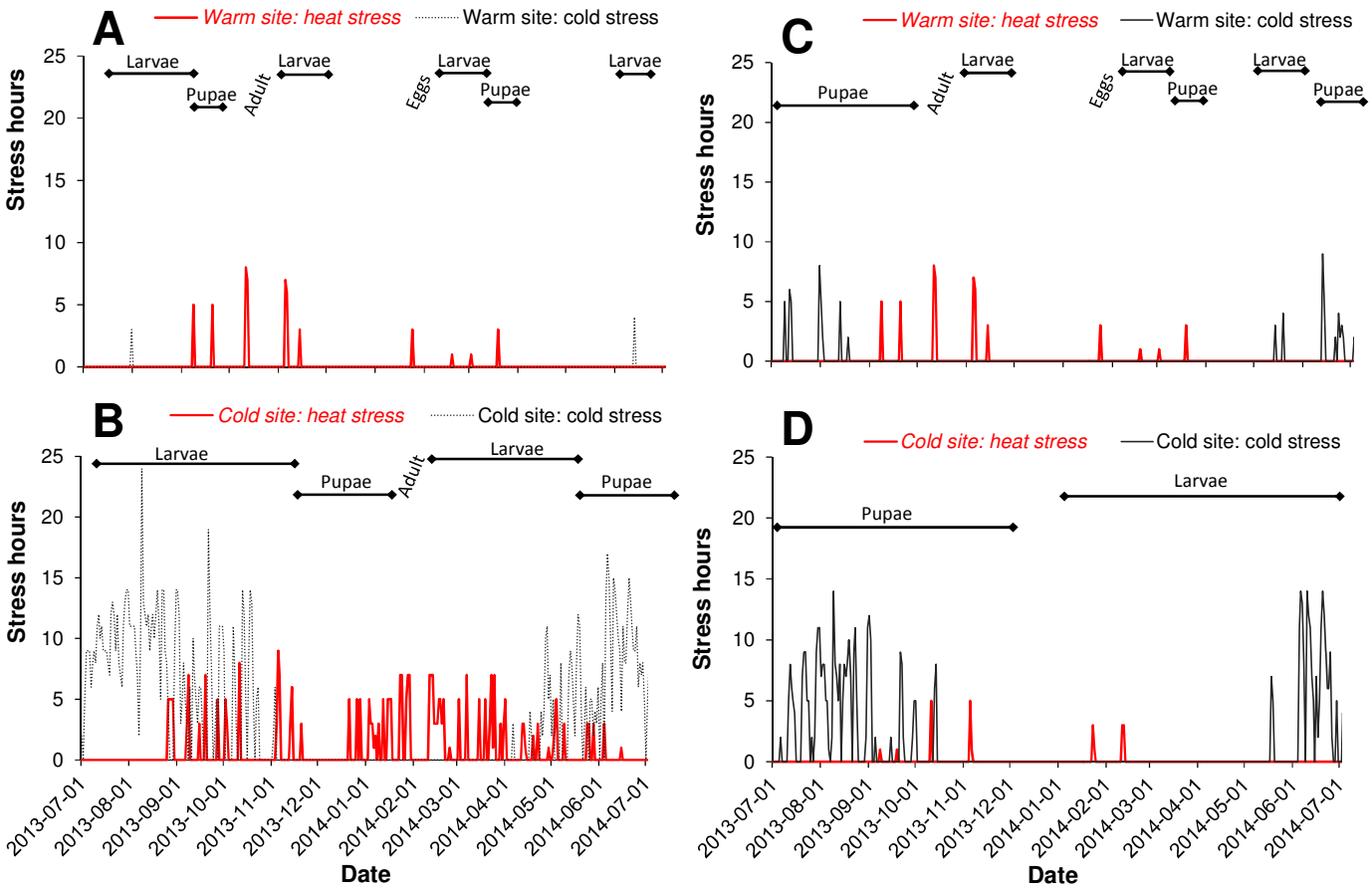

Figure 5.2 The impact of local climate and over-wintering stage on the number of stress hours for the specific life stages during the season. Model outcomes were based on A – B) larval and C – D) pupal over-wintering stages at a warm site and a cold site in South Africa. Horizontal bars denote the life stage relevant for stress hours observed.

The relative fitness of *E. saccharina* life-stages was significantly affected by the two sites (Table 5.2) so that relative fitness was higher at the warm site in comparison to the cold site (Figure 5.3 A, B). Relative adult abundance predictions for *E. saccharina* were also significantly affected by site and over-wintering life stage (Table 5.2, Figure 5.3 C, D). Female fecundity (number of eggs that could be produced per female per hour) was significantly affected by the over-wintering life stage and the site (Table 5.2), so that reproduction and/or egg laying commenced sooner at the warm site than at the cold site. Estimates of fecundity showed the first spikes during November at the cold site following pupal over-wintering in comparison to January (42 days later) following larval over-wintering. This lag effect between the over-wintering stages resulted in a predicted 13 552 more eggs that could be produced at the cold site following pupal over-wintering *vs.* larval over-wintering.

The availability of year-round in-field scout records enabled the verification of temporal model predictions. Here, model predictions for insect stress hours were plotted against scout records (expressed as average % larvae) in the field. Larval-overwintering, cold site model predictions were used for the plots because the larval scout data during winter revealed larval presence in the field and the scout data were obtained from the cold model site. Larval incidence was highest when the model predictions for insect stress in the environment were lowest and *vice versa* (Figure 5.4A). Furthermore, predictions for the adult stage corresponded with notable absences of larval observations in the scout records (Figure 5.4A), possibly further explaining population demographics observed in the field. In summary,

larval presence, according to the biophysical model, overlapped well with positive scout records averaged across a matrix of sugarcane field ages and cultivars (Figure 5.4B).

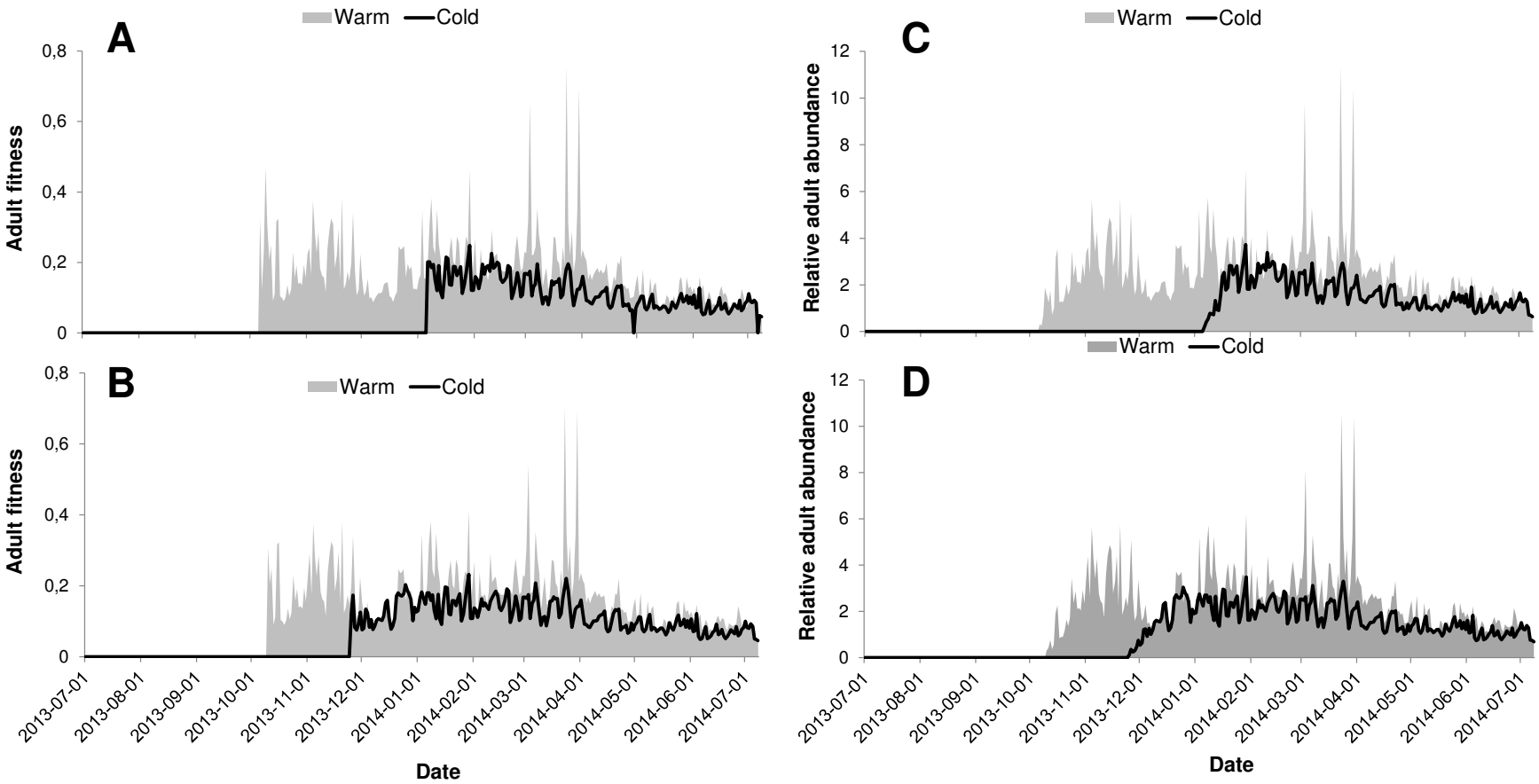

Figure 5.3 The impact of local climate and over-wintering stage on A & B) adult moth fitness and C & D) relative adult abundance. Model predictions were based on a A & C) larval and B & D) pupal over-wintering stage.

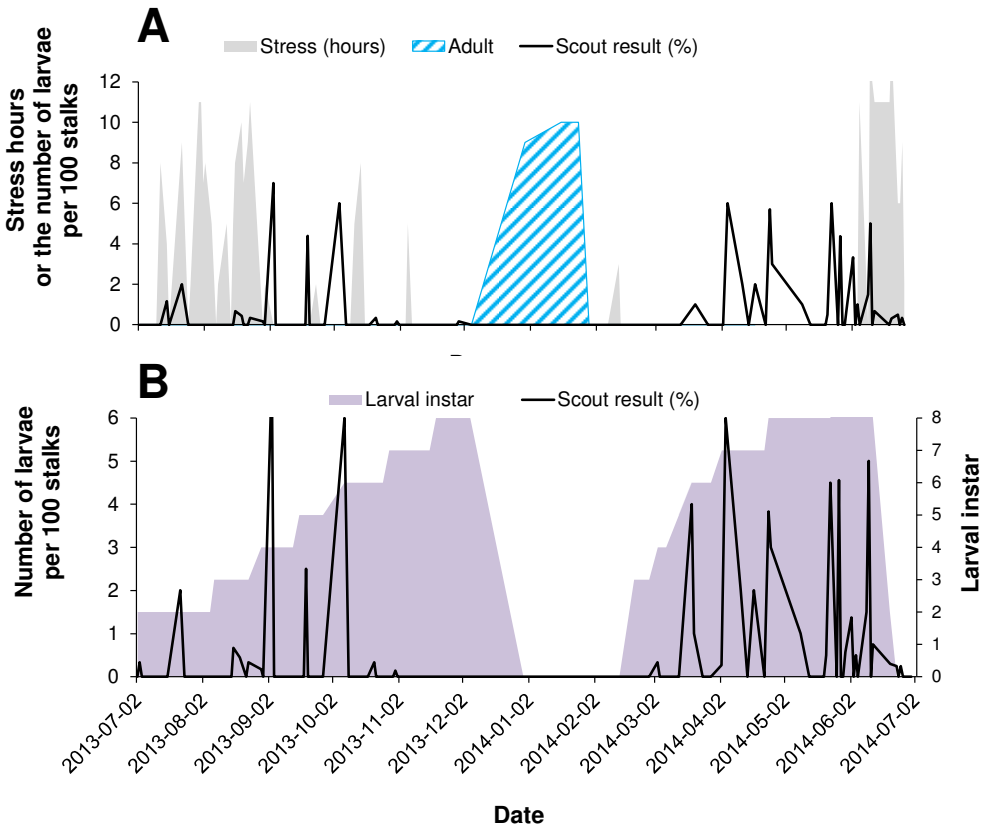

Figure 5.4 A) Biophysical thermal stress index (hours), timing of the adult life stage and B) larval instars over time (tallied for a larval over-wintering life stage) are compared to average scout records (expressed as a % larvae scouted) obtained from 29 sugarcane fields (ages 7 – 31 months, 16 different cultivars) in the Eston area (cold site: 29°52′00″S, 30°31′00″E, 785 m.a.s.l).

5.4 Conclusion

Modern integrated pest-management programs rely heavily on an understanding the pest phenology in order to develop predictive tools to plan timely interventions, especially with biological-control. Here I therefore aimed to develop a better understanding of the effect of local climate on *E. saccharina* population phenology and fitness, since it is clear that temperature, through its effect on insect life-cycle duration, forms a critical part of any control program. Locomotion performance, adult size, body mass, fecundity, growth rate and development time is temperature dependent and affects insect field fitness (Angilletta et al., 2002; Terblanche et al., 2006; Kingsolver & Huey, 2008). The sterile insect release program, will form part of the IPM plan against *E. saccharina* in the sugarcane industry (Conlong & Rutherford, 2009). However, to obtain maximum effect with minimum costs, sterile male moth releases should be conducted when the natural population of moths are low and in the initial stage of moth peak as mating ratios and released moth performance are critical aspects of SIT success (Chidawanyika & Terblanche, 2011; Sørensen et al., 2012; Terblanche, 2014). In this way, sterile-male mating will optimize sterile egg production during the wild moth's peak so that the effect on the population is maximised towards reproductive suppression. Here I provide support for adult moth abundance variation across geographic sites that depended on the over-wintering life-stage and local climatic conditions. I specifically showed that larval over-wintering led to a reduction in adult moth occurrence, three complete generations at a warm site and one generation and more frequent cold- and heat stress at a cold field site relative to the pupal overwintering scenario. Relative population fitness and abundance did not differ between the over-wintering stages, however fitness and the resulting abundance was significantly lower at the cold site. Field releases of sterile moths in warm sites can be done at the start of the first moth peak of the season, i.e. July – September, to bring down peak predicted to occur during October, irrespective of over-wintering stage. In cold sites, however, these results suggest that releases

should be delayed by approximately one month when the pupal stages are most abundant during winter-month scouting.

The model predictions and estimates of timing and abundance were compared to a large in-field scout dataset. Here the model performed relatively well. For example, the number of predicted stress hours was high, or the model projected an adult life stage which is not sampled in the scout data, during the times when larval scout data were zero. Model predictions for larval presence overlapped well with positive scout records averaged across a matrix of sugarcane ages and cultivars. Furthermore, earlier light trapping work showed that adult moth phenological cycles showed a small definite September peak (Carnegie & Leslie, 1990), peaks during November – December and March – May (Atkinson, 1982) and, specifically, in April (Carnegie & Leslie, 1990). Here, the model predictions, for both sites and over-wintering stages, correspond well to these findings. Furthermore, definite low adult moth abundance was recorded by Atkinson (1982) and Cernegie & Leslie (1990) during June – July, which the model reflected through an incomplete pupal period reaching the end of July. To conclude, the model corresponded well with adult moth peak events on the calendar, and larval abundance predictions were verified by on-farm scouting practices. In the broader context, these results hold important implications for field releases of SIT moths in different areas (Potgieter et al., 2013), and or biological control agents targeting different larval instar ages and/or pupae (Conlong, 1990; 1994). They can also be used to better predict insecticide applications targeting specific stages in the *E. saccharina* life cycle (Leslie, 2006). The application of this biophysical model can inform growers and extension staff to ensure accurate and timely management actions against this pest in the field.

Mathematical, mechanistic (process-based) population modelling enables scenario-driven hypothesis testing. Here overwintering life-stage significantly affected the generation time in the

cold, but not hot climates. Warm climates are more stable and temperatures fluctuated closer to the thermal optimum of the insects, resulting in fewer stress hours experienced, in both magnitude and frequency. Stress, in turn, resulted in changes in overall population abundance and estimated fitness. I showed that larvae experienced more stress in a cold environment following larval over-wintering in comparison to a warm site and pupal over-wintering. This suggests that, in cold environments, there might be selective pressure for *E. saccharina* to develop cold resistance during the larval stage or complete the final larval instar in order to pupate and overwinter in this stage.

The results obtained in this study are dependent on the incorporation of stage-specific trait data. This is not typically done for species abundance models, and the procedure outlined here is fairly unique and novel even to the pest modelling literature. There are however limitations to the model as it currently stands. For example, I modelled a single season to focus primarily on the effects of local climate and overwintering life-stage on the model predictions. When more than one season's phonological and abundance responses are captured, it will likely sketch a better picture about the long-term stressors that works on the species in a particular environment. From a broader point of view, sophisticated models that have been developed to predict the outcomes of IPM intervention (Potgieter et al., 2012; 2013) provide excellent insight into temporal population responses in the light of future pest management plans. Nevertheless, the mechanism-based model applied in this study provided novel insights into the effect of newly-invaded cold climates on *E. saccharina* survival and adult relative abundance. These results, in addition to many other observations concluding that *E. saccharina* will spread into novel areas, provide proof for complete generation turnover and population fitness in cold climates. This study also provides a good indication for more generations in warmer climates, which might therefore involve higher pest abundance and crop damage under global warming-type changes in climate. Higher average temperatures might reduce the number of cold stress events (e.g. freeze thaw cycles), accelerate the development times

and lead to range expansion into areas that are currently too cold for *E. saccharina* persistence. Management strategies should be tailored according to local climates, especially in areas where multiple generations are expected to occur and also in areas where the pest might expand its range.

The results provide valuable insights into the management of *E. saccharina* in commercially grown sugarcane. The predicted output parameters are directly relevant and interpretable to the general sugarcane grower in the specific site locations. It furthermore holds strong significance to the broader sugarcane industry through added insight into the effect of local climate and over-wintering stage on *E. saccharina* population abundance. This work should aid in decision making when IPM strategies are employed in the field.

5.5 References

Angilletta, M. J., Jr., Niewiarowski, P. H. & Navas, C. A. (2002) The evolution of thermal physiology in ectotherms. Journal of Thermal Biology 27: 249–268.

Assefa, Y., Mitchell, A. & Conlong, D. E. (2006) Phylogeography of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Annales de la Société Entomologique de France 42: 331–337.

Assefa, Y., Conlong, D. E., van den Berg, J. & Le Rü, B. P. (2008) The wider distribution of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa and its potential risk to maize production. Proceedings of the South African Sugar Technologists' Association 81: 290–297.

Assefa, Y., Conlong, D. E., van den Berg, J. & Mitchell, A. (2010) Distribution of sugarcane stem borers and their natural enemies in small-scale farmers' fields, adjacent margins and wetlands of Ethiopia. International Journal of Pest Management, 56: 233–241.

Atkinson, P. R. (1979) Distribution and natural hosts of *Eldana saccharina* Walker in Natal, its oviposition sites and feeding patterns. Proceedings of the South African Sugar Technologists' Association 53: 111–115.

Atkinson, P. R. (1980) On the biology, distribution and natural host plants of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Journal of the Entomological Society of Southern Africa 43: 171–194.

Atkinson, P. R. (1982) Phenology of *Eldana saccharina* Walker in Natal and use of light traps to monitor distribution and abundance. Proceedings of the South African Sugar Technologists' Association 56: 90–94.

Atkinson, P. R., Carnegie, A. J. M. & Smaill, R. J. (1981) A history of the outbreaks of *Eldana saccharina* Walker, in Natal. Proceedings of the South African Sugar Technologists' Association 55: 111–115.

Barton, M. G. & Terblanche, J. S. (2014) Predicting performance and survival across topographically heterogeneous landscapes: the global pest insect *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae). Austral Entomology 53: 249–258.

Briere, J. F. Pracros, P., Le Roux, A. Y. & Pierre, J. S. (1999) A novel rate model for temperature-dependent development for arthropods. Environmental Entomology 28: 22–29.

Butterfield, M. K. (2002) Genetic models to assess the development of counter-resistance in insect pests exposed to Bt-sugarcane. Proceedings of the South African Sugar Technologists' Association 76: 329–335.

Campbell, G. S. & Norman, J. M. (1998) An introduction of environmental biophysics, 2nd edition, Springer Science, New York, USA.

Cardwell, K. F., Schulthess, F., Ndemah, R. & Ngoko, Z. (1997) A systems approach to assess crop health and maize yield losses due to pests and diseases in Cameroon. Agriculture, Ecosystems and Environment 65: 33–47.

Carnegie, A. J. M. (1974) A recrudescence of the borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Proceedings of the South African Sugar Technologists' Association 48: 107–110.

Carnegie, A. J. M. & Leslie, G. W. (1990) *Eldana saccharina* (Lepidoptera: Pyralidae): ten years of light trapping. Proceedings of the South African Sugar Technologists' Association 64: 107–110.

Chidawanyika, F. & Terblanche, J. S. (2011) Costs and benefits of thermal acclimation for codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): implications for pest control and the sterile insect release programme. Evolutionary Applications 4: 534–544.

Chown, S. L., Slabber, S., McGeogh, M. A., Janion, C. & Leinaas, H. P. (2007) Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. Proceedings of the Royal Society B: Biological Sciences 274: 2661–2667.

Conlong, D. E. (2001) Biological control of indigenous African stemborers: What do we know? Insect Science and its Application 21: 267–274.

Conlong, D. E. (1990) A study of pest-parasitoid relationships in natural habitats: An aid towards the biological control of *Eldana saccharina* (Lepidoptera: Pyralidae) in sugarcane. Proceedings of the South African Sugar Technologists' Association 64: 111–115.

Conlong, D. E. (1994) A review and perspectives for the biological control of the African sugarcane stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Agriculture, Ecosystems and Environment 48: 9–17.

Conlong, D. E. & Rutherford, S. R. (2009) Conventional and New Biological and Habitat Interventions for Integrated Pest Management Systems: Review and Case Studies using *Eldana saccharina* Walker (Lepidoptera: Pyralidae). In: Integrated Pest Management: Innovation-Development Process (Peshin, R. & Dhawan, A. K. eds.), Vol. 1, Chapter 10, pp. 241–261. Springer, Dordrecht, The Netherlands. ISBN 978-1-4020-8991-6 (Print); 978-1-4020-8992-3 (online). DOI 10.1007/978-1-4020-8992-3_10.

Crawley, M. J. (2007) The R Book. John Wiley and Sons, Chichester.

Davis, P. M. & Padigo, P. (1990) Yield responses of corn stands to stalk borer (Lepidoptera: Noctuidae) injury imposed during early development. Journal of Economic Entomology 83: 1582–1586.

Dick, J. (1945) Some data on the biology of the sugarcane borer (*Eldana saccharina Wlk*). Proceedings of the South African Sugar Technologists' Association 19: 75–79.

Feng, H., Gould, F., Huang, Y., Jiang, Y. & Wu, K. (2010) Modeling the population dynamics of cotton bollworm *Helicoverpa armigera* (Hüber) (Lepidoptera: Noctuidae) over a wide area in northern China. Ecological Modelling 221: 1819–1830.

Girling, D. J. (1972) *Eldana saccharina* Wlk. (Lepidoptera: Pyralidae), a pest of sugarcane in East Africa. Proceedings of the International Society of Sugarcane Technologists 14: 429–434.

Girling, D. J. (1978) The distribution and biology of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) and its relationship to other stem borers in Uganda. Bulletin of Entomological Research 68: 471–488.

Goebel, F-R. & Way, M. (2006) Crop losses due to two sugarcane stem borers in Reunion and South African. Proceedings of the South African Sugar Technologists' Association 26: 806–812.

Huey, R. B. & Stevenson, R. D. (1979) Integrating thermal physiology and ecology of ectotherms: Discussions of approaches. American Zoologist 19: 357–366.

Kfir, R. (1998) Maize and grain sorghum: Southern Africa. In: African cereal stem borers. Economic importance, taxonomy, natural enemies and control (Polaszek, A. ed.), pp. 29-37. CAB International in association with ACP-EU Technical Centre for Agricultural and Rural Co-operation (CTA). Wallington, United Kingdom.

King, H., Conlong, D. E. & Mitchell, A. (2002) Genetic differentiation in *Eldana saccharina* (Lepidoptera: Pyralidae): Evidence from the mitochondrial cytochrome oxidase I and II genes. Proceedings of the South African Sugar Technologists Association 76: 321–328.

Kingsolver, J. G. & Moffat, R. J. (1982) Thermoregulation and the determinents of heat transfer in *Colias* butterflies. Oecologia 53: 27–33.

Kingsolver, J. G. & Huey, R. B. (2008) Size, temperature and fitness: three rules. Evolutionary Ecology Research 10: 251–268.

Kleynhans, E., Conlong, D. E. & Terblanche, J. S. (2014a) Host plant-related variation in thermal tolerance of *Eldana saccharina* (Lepidoptera: Pyralidae). Entomologia Experimentalis et Applicata 150: 113–122.

Kleynhans, E., Mitchell, K. A., Conlong, D. E. & Terblanche, J. S. (2014b) Evolved variation in cold tolerance among populations of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa. Journal of Evolutionary Biology 27: 1149–1159.

Lange, C. L., Scott, K. D., Graham, G. C., Sallam, M. N. & Allsopp, P. G. (2004) Sugarcane moth borers (Lepidoptera: Noctuidae and Pyraloidae): Phylogenetics constructed using COII and 16S mitochondrial partial gene sesquences. Bulletin of Entomological Research 94: 457–464.

Leslie, G. W., Stranack, R. A. & De Haas, O. (2006) Progress in the use of aerially applied fastac® (alpha-cypermethrin) for the control of the sugarcane borer *Eldana saccharina* (Lepidoptera:

Pyralidae), and an assessment of its commercial impact. Proceedings of the South African Sugar Technologists' Association 80: 236–244.

Maes, K. V. N. (1998) Pyraloidea: Crambidae, Pyralidae. In: African cereal stem borers. Economic importance, taxonomy, natural enemies and control (Polaszek, A. ed.), pp. 87–98. CAB International in association with ACP-EU Technical Centre for Agricultural and Rural Co-operation (CTA). Wallington, United Kingdom.

Potgieter, L., van Vuuren, J. H. & Conlong, D. E. (2012) Modelling the effects of the sterile insect technique applied to *Eldana saccharina* Walker in sugarcane. ORiON 28: 59–84.

Potgieter, L., van Vuuren, J. H. & Conlong, D. E. (2013) A reaction-diffusion model for the control of *Eldana saccharina* Walker in sugarcane using the sterile insect technique. Ecological Modelling 250: 319–328.

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3–900051–07–0, URL http://www.R–project.org/.

Sørensen, J. G., Addison, M. F. & Terblanche, J. S. (2012) Mass-rearing of insects for pest management: challenges, synergies and advances from evolutionary physiology. Crop Protection 38: 87–94.

Terblanche, J. S. (2014) Physiological performance of field-released insects. Current Opinion in Insect Science. *In Press.* DOI: 10.1016/j.cois.2014.06.003

Terblanche, J. S., Klok, J. C., Krafsur, E. S. & Chown, S. L. (2006) Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (diptera: Glossinidae): implications for distribution modelling. The American Journal of Tropical Medicine and Hygiene 74: 786–794.

Waiyaki, J. N. (1968) The biology and control of the principal lepidopterous borers associated with sugarcane at the Tanganyika Planting Company, Arusha-Chini. Miscellaneous report No. 653. Tropical Pesticides Research Institute, Arusha, Tanzania.

Watt, W. B. (1968) Adaptive significance of pigment polymorphisms in *Colias* Butterflies. I. Variation of melanin pigment in relation to thermoregulation. Evolution 69: 1486–1496.

Way, M. J. (1994) A preliminary assessment of the effects of different constant temperatures on the reproduction of *Eldana saccharina* (Lepidoptera: Pyralidae). Proceedings of the South African Sugar Technologists' Association 68: 16–18.

Way, M. J. (1995) Developmental biology of the immature stages of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Proceedings of the South African Sugar Technologists' Association 69: 83–86.

Webster, T. M., Maher, G. W. & Conlong, D. E. (2006) An integrated pest management system for *Eldana saccharina* in the midlands north region of KwaZulu-Natal. Proceedings of the South African Sugar Technologists' Association 79: 347–358.

Webster, T. M., Brenchley, P. G. & Conlong, D. E. (2009) Progress of the area-wide integrated pest management plan for *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in the midlands north region of KwaZulu-Natal. Proceedings of the South African Sugar Technologists' Association 82: 471–485.

Chapter 6

Synthesis and general discussion

Temperature has a marked effect on a range of insect physiological and life-history processes. First, understanding tolerance of thermal extremes by pest insects is essential for developing integrated management strategies for these, as tolerance traits can provide insights into constraints on their activity and survival. A major question in thermal biology is whether thermal limits vary systematically with microclimate variation, or whether other biotic or abiotic factors can influence these limits in a predictable manner.

Chapter 2 reports the results of experiments determining thermal limits to activity and survival of *E. saccharina* collected from either sugarcane or *C. papyrus*. The physiological measurements were taken after rearing under standard conditions in the laboratory for 1 – 2 generations. CT_{min}, CT_{max}, LLT, and freezing temperature were compared between *E. saccharina* collected from the two host plants. CT_{min} and CT_{max} of *E. saccharina* moths collected from sugarcane were significantly lower than those from *C. papyrus* (CT_{min} = 2.8 ± 0.4 *vs.* 3.9 ± 0.4 °C; CT_{max} = 44.6 ± 0.1 *vs.* 44.9 ± 0.2 °C). By contrast, LLT of moths and freezing temperatures of pupae did not vary with host plant (LLT for 50 % [LT_{50}] of the moth population when collected from sugarcane: -3.2 ± 0.5 °C, from *C. papyrus*: -3.9 ± 0.8 °C; LT_{90} from sugarcane: -10.0 ± 0.9 °C, from *C. papyrus*: -8.9 ± 0.8 °C). Freezing temperatures of pupae collected from *C. papyrus* were -18.0 ± 1.0 °C and of those from sugarcane -17.5 ± 1.8 °C. The moths that experienced the lowest minimum temperature (in *C. papyrus*) did not have the lowest CT_{min}, although the highest estimate of CT_{max} was found in *E. saccharina* moths collected from *C. papyrus* and this was also the microsite that recorded the highest maximum temperatures. These results therefore suggest that host plant may strongly mediate lower critical thermal limits, but not necessarily LLTs or freezing temperatures.

As part of an area-wide integrated pest management (AW-IPM) plan for *E. saccharina* in sugarcane, habitat management (or a 'push-pull' strategy) is advocated (Conlong & Rutherford,

2010). The planting of *C. papyrus* into wetland areas where it once occurred naturally is encouraged because of the plant's positive attractiveness over sugarcane to *E. saccharina* (Conlong et al., 2007), and the presence of a number of natural parasitoid enemies of *E. saccharina* larva, present in the former host plant, but not in the latter (Conlong, 1990) . This preference of *E. saccharina* for its indigenous host plants reduces its population in sugarcane, thereby also reducing the numbers of sterile moths needed for release in a sterile insect release program, also part of the AW-IPM approach (Conlong & Rutherford, 2010). The increased thermal biology knowledge of *E. saccharina* from its two host plant sources, as demonstrated in this paper, thus allows the refining of source population collections (best to collect from sugarcane) of *E. saccharina* so that laboratory-reared individuals are as competitive, i.e. can tolerate low temperatures, as individuals from field populations, especially with respect to low-temperature performance (see, e.g., discussion in Sørensen et al., 2012). The ability to keep these thermal trait responses constant after several-generation rearing in the laboratory are however not known, and this were studied in part in Chapter 3 of this thesis.

Physiological responses to a particular micro-environment providing alternative nutritional values and non-buffered microclimates (such as *C. papyrus*) should be further examined to better understand the persistence of *E. saccharina* in the wild. Moreover, further knowledge of biotic interactions associated with the host plants (Ferrari et al., 2004; Karley et al., 2004; Dunbar et al., 2007) may be a useful avenue for further work. Nutritional characteristics associated with different host plants might affect short-term physiological responses and survival through their impact on thermal physiology of *E. saccharina*. However, it is important to note that Graham (1990) found that there was no significant difference between the body weight of pupae which had developed on an artificial diet and pupae developing naturally in sugarcane or *C. papyrus*. The mean ± SD weight

of female and male pupae was 153 ± 27 mg and 81 ± 19 mg from artificial diet, 126 ± 26 mg and 83 ± 29 mg from sugarcane, and 155 ± 36 mg and 90 ± 16 mg from *C. papyrus* respectively.

The results from Chapter 2 have significant implications for on-going pest management and thermal biology of these and other insects that occur naturally in diverse micro-habitats. For example, Pelini et al., (2009) showed that range expansion were dependent on wild host-plant availability, while the environmental effect of the specific host on physiological outcomes cannot be ignored (Buckley & Kingsolver, 2012). Interactions between insects and host plants, as well as the variation in microclimates caused by host plants remain important (Pincebourde & Woods, 2012). Here I learned that *E. saccharina* from sugarcane will probably show less cold stress due to higher cold tolerance in comparison to moths in the wild host plant, *C. papyrus*. Further work is needed to understand the physiological mechanisms involved that drives higher cold tolerance in sugarcane which might also have significant implications for on-going pest management and the thermal biology of this pest.

In Chapter 3, using a common-garden approach, I show pronounced variation in chill coma onset temperature (± 4 °C) across the geographic range of *E. saccharina* in South Africa. Variation in CT_{min} was significantly positively correlated with mean minimum temperature of geographic collection sites, suggesting a stable association with local microclimatic conditions. Crosses between the most and least cold-susceptible geographic lines confirmed a genetic component to CT_{min} trait variation. Slower developmental time in the most low-temperature tolerant population suggests this lower CT_{min} adaptation has come at a cost to fitness, but allows greater survival and activity in that environment. A significant reduction of phenotypic plasticity in the laboratory population suggests that plasticity of this trait is costly to maintain but also necessary for survival in natural environments.

Among-population variation in chill coma induction or onset temperature is thought to reflect natural selection for local microclimatic conditions. However, many insect species exhibit overwintering strategies that may reduce exposure to extreme cold temperatures in susceptible life stages, thus reducing the evolutionary impact of cold stress on their distributions. As such, few studies have investigated the evolutionary importance of cold tolerance limits in natural populations. The outcomes of Chapter 3 provide two notable results in the context of evolved chill coma variation: 1) CT_{min} differs significantly between geographic lines and is significantly positively correlated with local climates after accounting for a range of potential confounding factors, and 2) there is a stable genetic architecture underlying CT_{min} trait variation, likely representing four key genes. These results are significant to understanding evolved variation in CT_{min} among populations and also have significant pest management implications, such that one cannot assume that the low temperature tolerance remains constant throughout the geographic distribution of *E. saccharina* populations, while further work is needed to dissect the underlying mechanism that drives the observed physiological trait variation and gene(s) responsible for thermal trait variation among populations.

Phenotypic responses affect evolutionary consequences for populations, and from Chapter 4 the data suggests that *E. saccharina* employs adjustments of physiological water balance-traits to cope with temperature variation during development (see also Price et al., 2003; Helmuth et al., 2005). *E. saccharina* can acclimate physiologically within a single generation, during immature life stages, and alter their adult water balance physiology (see Huey et al., 1999) and low temperature tolerance (Chapter 3). The ability of *E. saccharina* to adapt to environmental change within a short time frame shows support for functional, phenotypic change which is probably also underlying genetically evolved changes to cope with warmer conditions (e.g. Cooper et al., 2010). It appears that acclimation to warmer conditions enhanced fitness (survival time) under desiccating conditions

as adults, while sub-optimal trait responses (high WLRs and low BWC_{CRIT} associated with cold conditions) led to potential fitness costs (Hoffmann, 1995; Deere & Chown, 2006; Terblanche & Kleynhans, 2009).

For water balance physiology, prior thermal history may pre-condition individuals to be more sparing in their water consumption at a given temperature upon subsequent exposure, or alternatively, may relax constraints on water economy leading to more frivolous use of water at a later stage. In Chapter 4, I tested these two major alternative hypotheses on the adult life stage of *E. saccharina* by exposing them to different rearing temperatures during development and comparing adult physiological performance (water loss rates, time to death) and water-balance related traits (body size, water content). Developmental acclimation at 20.0 °C, 25.0 °C or 30.0 °C throughout the larval and pupal stage resulted in significant effects on water balance traits of two-day old adult male and female *E. saccharina*. In summary, lower developmental acclimation resulted in a 61 % increase in water loss rate (range: 0.78 mg/h) and a 26 % reduction in survival time (6.8 h). Initial body water content and initial body mass remained similar across male acclimation groups while higher developmental acclimation reduced female body mass significantly. High developmental acclimation resulted in significantly higher (~ 23 %) body water content at death, possibly indicating a better overall ability to withstand desiccating conditions, although there was no difference in time to death compared to the intermediate group. The relationship between time to death and body mass was altered from negative at 25 °C and 30 °C acclimation, to positive at 20 °C acclimation. These results show fundamental and pervasive effects of rearing temperature on adult physiological performance, with low temperature generally relaxing what appear to be substantial constraints on water economy at higher temperatures for *E. saccharina*. Furthermore, they are significant for understanding the recent range of expansion of *E. saccharina* into cooler environments in southern Africa and for management of the species, because, larvae experienced

more stress in a cold environment following larval over-wintering in comparison to a warm site and pupal over-wintering, suggesting that, in cold environments, there might be selective pressure for *E. saccharina* to develop cold resistance during the larval stage or complete the final larval instar in order to pupate and overwinter in this stage. The former, i.e. enhanced cold resistance, will possibly result in range expansion to even colder conditions in the sugar belt. Hotter winter climates from one growing season to a next will result in higher pest population pressures, especially in sugarcane that are susceptible already for infection (older or carry-over cane) (see Webster, 2006).

Due to the variation in life stage sensitivity toward climate, agricultural insect pest models are often not easily interpreted or lack relevance to management strategies in the field. In Chapter 5 a biophysical model that incorporates climate data with life stage dependent physiology and predict *E. saccharina* life stage- and generation turnover, cold and heat stress, relative fitness and -abundance in the field were employed. The aim of this Chapter was to establish whether over-wintering stage and local climate significantly affected the population responses of *E. saccharina* in the field. The results showed that larval over-wintering led to reduce adult moth occurrence at both the warm and cold site, three generations at a warm site and one generation, more frequent cold- and heat stress and a 42-day lag which resulted in a prediction of 13 552 eggs produced less, at a cold site. Relative fitness did not differ between the over-wintering stages, however fitness of both stages was lower at the cold site. The model estimates were compared to and interpreted within the context of a large scout dataset. The number of predicted stress hours was high or the model projected an adult life stage during the times when field scout data were zero, suggesting that high stress correlated with low field observation. Larval presence overlapped well with positive scout larval records averaged across a matrix of sugarcane ages and cultivars. The results are important for integrated pest management strategies in a way that management strategies should be tailored

according to local climates, especially in areas where multiple generations are expected to occur and also in areas where the pest might expand its range.

To close, management recommendations to the sugarcane industry include careful planning when different host- and climatic sites are considered for *E. saccharina* collection or SIT moth releases. Future work include looking at the combination of geographic climate variation, local population physiology and SIT efficacy on wild *E. saccharina* population numbers. The metabolic- and genetic mechanisms that is involved in altering thermal physiological trait responses across micro- and macro-climatic ranges respectively also needs further attention, whilst the ability of *E. saccharina* to adapt and change over short (however not excluding long-term evolutionary changes) time-scales should be considered in rearing practices and for future SIT moth release plans.

6.1 References

Conlong, D. E. (1990) A study of pest-parasitoid relationships in natural habitats: an aid towards the biological control of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in sugarcane. Proceedings of the South African Sugar Technologists' Association 64: 111–115.

Conlong, D. E. & Rutherford, R. S. (2009) Conventional and new biological and habitat interventions for integrated pest management systems: review and case studies using *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Integrated Pest Management: Innovation-Development Process (ed. by R Peshin & AK Dhawan), pp. 241–261. Springer, Dordrecht, The Netherlands.

Conlong, D. E., Kasl, B. & Byrne, M. (2007) Independent kids or motherly moms? Implications for integrated pest management of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Proceedings of the International Society of Sugar Cane Technologists 26: 787–796.

Cooper, B. S., Czarnoleski, M. & Angilletta Jr., M. J. (2010) Acclimation of thermal physiology in natural populations of *Drosophila melanogaster*: a test of an optimality model. Journal of Evolutionary Biology 23: 2346–2355.

Deere, J. A. & Chown, S. L. (2006) Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. The American Naturalist 168: 630–644.

Dunbar, H. E., Wilson, A. C. C., Ferguson, N. R. & Moran, N. A. (2007) Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. PloS Biology 5: 1006–1015.

Ferrari, J., Darby, A. C., Daniell, T. J., Godfray, H. C. J. & Douglas, A. (2004) Linking the bacterial community in pea aphids with host-plant and natural enemy resistance. Ecological Entomology 29: 60–65.

Graham, D. Y. (1990) Development of mass rearing methods for *Eldana saccharina* (Lepidoptera: Pyralidae) I: control of fungal contamination on the artificial diet. *Proceedings of the South African Sugar Technologists' Association* 64: 116–119.

Helmuth, B., Kingsolver, J. G. & Carrington, E. (2005) Biophysics, physiological ecology, and climate change: does mechanism matter? Annual Review of Entomology 67: 177–201.

Hoffmann, A. A. (1995) Acclimation: increasing survival at a cost. Trends in Ecology and Evolution 10, 1–2.

Huey, R. B., Berrigan, D., Gilchrist, G. W. & Herron, J. C. (1999) Testing the adaptive significance of acclimation: a strong inference approach. American Zoology 39: 323–336.

Karley, A. J., Parker, W. E., Pitchford, J. W. & Douglas, A. E. (2004) The mid-season crash in aphid populations: why and how does it occur? Ecological Entomology 29: 383–388.

Price, T.D., Qvarnström, A. & Irwin, D.E. (2003) The role of phenotypic plasticity in driving genetic evolution. Proceedings of the Royal Society B: Biological Sciences 270: 1433–1440.

Sørensen, J. G., Addison, M. F. & Terblanche, J. S. (2012) Mass-rearing of insects for pest management: Challenges, synergies and advances from evolutionary physiology. Crop Protection 38: 87–94.

Terblanche, J. S. & Kleynhans, E. (2009) Phenotypic plasticity of desiccation resistance in *Glossina* puparia: are there ecotype constraints on acclimation responses? Journal of Evolutionary Biology 22: 1636–1648.

Webster, T. M., Maher, G. W. & Conlong, D. E. (2006) An integrated pest management system for *Eldana saccharina* in the midlands north region of KwaZulu-Natal. Proceedings of the South African Sugar Technologists' Association 79: 347–358.