Clinal variation in life-history traits of the invasive plant species *Echium plantagineum* L.

Tara KonarzewskiSchool of the Environment



Thesis submitted for the degree of

Doctor of Philosophy

at the University of Technology, Sydney

October 2012



Certificate of Authorship/Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student

Acknowledgments

My thanks and heartfelt appreciation go to all those who helped me throughout this project. First and foremost, I would like to express my deep gratitude to my supervisors, Robert Godfree at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Brad Murray at the University of Technology, Sydney (UTS). Thank you for your endless enthusiasm, guidance, constructive feedback and thought-provoking discussions. I cannot thank you enough for taking the time help me with this project and it could not have succeeded without you.

This work would not have been possible without financial support from the University of Technology, Sydney, School of the Environment, Climate Change Cluster and the CSIRO Climate Change Flagship who supported me throughout my PhD. Facilities and funding for this research was provided by CSIRO Plant Industry, the CSIRO Climate Change Flagship and the University of Technology, Sydney. I would like to extend a special thank you to the staff of the CSIRO Plant Phenomics Facility and the CSIRO Black Mountain Analytical Services Team, especially Bob Furbank, Xavier Sirault and Nell Peisley for their assistance with sample processing, training, assistance with analyses and loan of equipment.

I would like to thank everyone who assisted me in the field and the glasshouse. In particular, I wish to thank the following people for their valuable help and assistance during the project: Leslie Weston for her assistance with sample identification and many valuable discussions regarding the study species, David Marshall for his expertise and assistance during glasshouse experiments and Carole Elliot for her knowledge and assistance with hand pollination techniques. I would like to thank the Beekeepers association of the Australian Capital Territory (ACT) for the loan of two hives of European honey bees (*Apis Mellifera*) during the pollination trial

and Tim Heard at the CSIRO, Ecosystem Services, Brisbane for the loan of a native stingless bee hive (*Trigona Carbonaria*).

There are times in your life when you truly understand how important it is to have supportive friends, I have been very lucky to have met and known some amazing people throughout the past few years. Thank you to my roommates, Tara Hopley and Carole Elliott for your support, confidence, helpful discussions and endless cups of tea that always made the world brighter. Thank you to Amanda Baker, Jessica Calnan and Amy McLeod for discussions, advice, support and welcome distractions. A special thank you to Megan Phillips for her friendship and support throughout this project. We spent many hours bouncing ideas and discussing issues. Her beautiful smile and cheerful attitude was an inspiration and without her encouragement I may not have made it this far.

Finally I would like to thank my family. To my sisters, Claire and Lianna Morgan, thank you for your assistance with field work, support, patience and understanding throughout the project. I am very grateful to my father, John Morgan, and my husband, Michael Konarzewski, for their invaluable proof-reading skills, helpful discussions, love, support and encouragement throughout the project. I would also like to extend a special thank you to my mother, Sue Morgan, for her love, support, dedication and encouragement. I have a deep admiration for both of my parents who are both world leaders in their respective fields and have also been able to raise a family. I hope to be lucky enough to one day to follow in their footsteps.

Abstract

Range expansion during the invasion of a novel environment requires that invading species adapt to geographical variation in climate and maintain positive population growth in the face of environmental heterogeneity. Thus, invasive species are expected to undergo adaptive evolutionary changes as they encounter novel selection pressures. The aim of this thesis was to identify adaptive changes in plant growth and reproductive traits throughout the lifecycle of a model invasive species to determine which traits are vital to the success of invasive species encountering novel environments.

The model species used in this study was the widespread European invader, *Echium plantagineum*, which has invaded over 33 million ha across Australia, causing ~\$30 million (AUD) damage per annum. I investigated geographic variation in life-history traits of 34 populations of *E. plantagineum* across a 1,000 km arid-mesic gradient throughout south-eastern NSW, Australia. Seeds were collected for each population along the arid-mesic gradient, germinated in the laboratory and grown in the glasshouse in a common environment.

I found that *E. plantagineum* has rapidly adapted to environmental selection pressures throughout its range, resulting in two major clines linked to plant flowering time and seed size of progeny. Compared with populations from mesic habitats along the arid-mesic gradient, plant populations from arid environments had significantly higher relative growth rate and leaf production which was associated with much earlier flowering time and reduced time between stem production and flower production. Plants from arid regions also produced significantly larger seeds compared with plants from mesic habitats. Interestingly, seeds from all *E*.

plantagineum populations along the arid-mesic gradient germinated rapidly (within 48 hours of water exposure) allowing them to quickly and opportunistically take advantage of available resources. Considered together, these adaptations allow *E. plantagineum* to grow rapidly, reproduce and produce progeny before conditions become unsuitable.

The findings in this thesis provide compelling evidence for the rapid development, within 150 years, of clines in reproductive strategies linked to flowering and seed size evolution. My results support the notion that the successful invasive spread of species can be increased through genetic divergence of populations along arid-mesic climatic gradients. The climate of south-eastern Australia is predicted to change to become hotter and drier inducing many species to adapt or perish. The range and distribution of *E. plantagineum* is unlikely to be altered by these climatic changes as pre-adapted genotypes currently exist in the range margins and have persisted in arid regions for over 100 years. Consequently, further work is required to investigate the evolutionary capacity of other native and invasive species to determine how ecosystem dynamics and composition may change in the future.

Table of Contents

Certificate of Authorship/Originality	ii
Acknowledgments	iii
Abstract	V
Table of Contents	vii
List of Figures	X
List of Tables	XV
Acronyms	.xvii
Chapter 1: Introduction	1
1.1 Biological invasions	1
1.2 Clinal differentiation of populations during invasion in response to climate .	2
1.2.1 Clinal patterns	2
1.2.2 Evolutionary underpinnings of clinal patterns.	6
1.2.3 Clinal patterns in a vulnerable environment: Arid environments	7
1.2.4 An experimental approach for determining clinal patterns	9
1.3 Invasive exotic plant species in Australia	10
1.3.1 Background	10
1.3.2 Echium plantagineum (Paterson's Curse): model study species	11
1.3.3 General biology of <i>Echium plantagineum</i>	11
1.3.4 History of introduction of <i>Echium plantagineum</i> to Australia	12
1.4 Research Approach	15
1.4.1 Thesis Structure	15
Chapter 2: A comparison of seed germination strategies among population Echium plantagineum along an arid-mesic gradient	
2.1 Introduction	17
2.1.1 Chapter aim and approach	20
2.2 Methods	21

2.2.1 Site selection and seed collection	21
2.2.2 Seed collection and measurement	24
2.2.3 Seed germination	25
2.2.4 Soil collection and nutrient analysis	26
2.2.5 Climate data and site conditions	26
2.2.6 Statistical analysis	27
2.3 Results	32
2.4 Discussion	36
Chapter 3: An examination of growth trait variation amo Echium plantagineum along an arid-mesic gradient	
3.1 Introduction	44
3.1.1 Chapter aim and approach	48
3.2 Methods	49
3.2.1 Seed germination and glasshouse conditions	49
3.2.2 Growth traits	50
3.2.3 Stature traits	54
3.2.4 Water use strategy	55
3.2.5 Climate, spatial and edaphic data collection	56
3.2.6 Statistical analysis	57
3.3 Results	58
3.3.1 Growth traits	58
3.3.2 Stature traits	67
3.3.3 Water use traits	75
3.4 Discussion	76
3.4.1 Observation of new defence mechanism	80
Chapter 4: Variation in reproductive traits of <i>Echium plantag</i> along an arid-mesic gradient	
4.1 Introduction	

4.1.1 Chapter aim and approach	87
4.2 Methods	88
4.2.1 Statistical analysis	90
4.3 Results	91
4.4 Discussion	104
Chapter 5: Rapid development of adaptive, climate-driven clinal variated mass in the invasive annual forb <i>Echium plantagineum</i> L	
5.1 Introduction	110
5.2 Methods	113
5.2.1 Seed collection and field sites	114
5.2.2 Common garden glasshouse experiment	115
5.2.3 Seed measurements	117
5.2.4 Site characterisation	117
5.2.5 Statistical analyses	120
5.3 Results	122
5.4 Discussion	127
Chapter 6: Discussion and synthesis	133
6.1 Overview of important findings	133
6.1.1 Summary of major results and conclusions	134
6.2 Implications for the ecology of <i>Echium plantagineum</i> in Australia?	137
6.2.1 Evidence for evolution since introduction	137
6.2.2 Response to arid environments	138
6.3 Implications of climate on the distribution and management	139
6.3.1 Management implications for <i>E. plantagineum</i>	140
6.3.2 Adjusting management programs for potential evolution	141
6.4 Key areas for future research	142
6.5 Final conclusion	146
References	148

List of Figures

Figure 3.2 Water use efficiency trial (a) Polyethylene beads added to the surface of pots to prevent water loss, (b) water use trial set-up with control pots covered in beads in between experimental plants
Figure 3.3 Relative growth rate measured over three time intervals 4-6, 6-8 and 8-12 weeks during a common garden glasshouse study; (a) actual means \pm SE; (b) RGR first harvest site level scores on PC1 and (c) RGR third harvest site level scores on PC1. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names. Bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).
Figure 3.4 Leaf number was measured over four time intervals four, six, eight and 12 weeks during a common garden glasshouse study; (a) actual means \pm SE; (b) leaf number first harvest site level scores on PC1 and (c) leaf number third harvest site level scores on PC1. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.
Figure 3.5 Leaf dimensions measured during common garden glasshouse study measured during four time intervals four, six, eight and 12 weeks; (a) largest leaf length actual means \pm SE; (b) largest leaf width actual means \pm SE; (c) largest leaf width fourth harvest compared to site level scores on PC2. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.
Figure 3.6 Leaf traits measured during common garden glasshouse study measured during four time intervals four, six, eight and 12 weeks; (a) specific leaf area (SLA) actual means \pm SE; (b) leaf area ratio (LAR) actual means \pm SE; (c) SLA third harvest site level scores on PC2. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.
Figure 3.7 Leaf accumulation rate (actual means \pm SE) measured during common garden glasshouse study measured during four time intervals four, six, eight and 12 weeks. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names65
Figure 3.8 Leaf nutrient content measured once during a common garden glasshouse study; (a) leaf nitrogen content (±SD); (b) leaf carbon content (±SD) and (c) leaf chlorophyll content (±SD). Abbreviations denote the individual bioregions, see Fig. 2.1 for full names. Bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST)
Figure 3.9 Petiole length and plant height measured over two harvest sessions eight and 12 weeks during a common garden glasshouse study; (a) plant height actual means \pm SE; (b) height at eight weeks site level scores on PC1; (c) plant petiole length of an average leaf actual means \pm SE. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.
Figure 3.10 Photosynthetic area (P_{area}) was measured over four time intervals four, six, eight and 12 weeks during a common garden glasshouse study; (a) P_{area} actual

means \pm SE; (b) P_{area} third harvest site level scores on PC1. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names
Figure 3.11 Above ground biomass (AGB) was measured over four time intervals four, six, eight and 12 weeks during a common garden glasshouse study; (a) AGE actual means ± SE; (b) AGB first harvest site level scores on PC1 and (c) AGB third harvest site level scores on PC1. Abbreviations denote the individual bioregions; Fig 2.1 for full names.
Figure 3.12 Below ground biomass (BGB) was measured over four time intervals four, six, eight and 12 weeks during a common garden glasshouse study; (a) BGE actual means \pm SE; (b) BGB first harvest site level scores on edaphic factors (PC2) Abbreviations denote the individual bioregions, see Fig. 2.1 for full names73
Figure 3.13 Root : shoot ratio (RSR) was measured over four time intervals four, six eight and 12 weeks during a common garden glasshouse study; (a) RSR actual means ± SE; (b) RSR first harvest site level scores on edaphic factors (PC2). Abbreviations denote the individual bioregions, see Fig. 2.1 for full names
Figure 3.14 <i>Echium plantagineum</i> plants with red trichomes, raised bumps on the leasurface, predicted to be used in defence against insects; (a) leaf cross section with trichome; (b) trichomes on leaf edge and (c) trichomes on leaf surface
Figure 4.1 Time to first stem initiation (SI_{time}) across the six bioregions; (a) actual means \pm SE; (b) adjusted Means \pm SE and least square mean post-hoc test. Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names. Bioregions are arranged from most westerly (BHC) to most easterly (COAST).
Figure 4.2 Time to first stem initiation (SI _{time}) across the six bioregions; cumulative curves of proportion of plants with stems over time (days from germination) Abbreviations denote the individual bioregions, see Fig. 2.1 for full names94
Figure 4.3 Time to first flower (FI_{time}) during a common garden glasshouse trial with <i>Echium plantagineum</i> averaged across the six bioregions; (a) actual means \pm SE; (b) adjusted means \pm SE. Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names 96
Figure 4.4 Relationships between flower initiation (FI _{time}) and two key predictor variables; (a) site-level scores on PC1 and (b) site longitude
Figure 4.5 Time to first flower (FI _{time}) during a common garden glasshouse trial with <i>Echium plantagineum</i> averaged across the six bioregions; cumulative curves of proportion of plants with flowers over time (days from germination). Abbreviations denote the individual bioregions, see Fig. 2.1 for full names

Figure 4.6 Time from first stem to first flower (SF_{time}) averaged per bioregion during a common garden glasshouse trial; (a) actual means \pm SE; (b) adjusted means \pm SE. Means sharing the same letter did not differ significantly at the 0.05 level PC2. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names. Bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).
Figure 4.7 Time from first stem to first flower (SF_{time}) averaged per bioregion during a common garden glasshouse trial; (a) Relationship between SF_{time} and site-level scores on PC1 and (b) Relationship between SF_{time} and site-level scores on PC2 100
Figure 4.8 Average photoperiod at first flower averaged per bioregion during a common garden glasshouse trial; (a) actual means \pm SE; (b) adjusted means \pm SE. Means sharing the same letter did not differ significantly at the 0.05 level Abbreviations denote the individual bioregions, see Fig. 2.1 for full names, bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST); and (c) Relationship between photoperiod and site-level scores on PC1101
Figure 4.9 Number of stems per plant averaged per bioregion during common garden glasshouse trial; (a) actual means \pm SE and (b) adjusted means \pm SE. Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names, bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST)
Figure 4.10 Average estimated total flower production per plant averaged per bioregion during a common garden glasshouse trial; (a) actual means \pm SE and (b) adjusted means \pm SE. Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names, bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).
Figure 5.1 Climatological data for the May-October growing season for bioregions in the study area. (a) Total precipitation (mm). (b) Mean temperature ($^{\circ}$ C). (c) Aridity Index. Abbreviations denote the individual bioregions, Abbreviations denote the individual bioregions, See Fig. 2.1 for full names. Bioregions are arranged along the x axis from most westerly (BHC) to most easterly (COAST)
Figure 5.2 Seed mass derived from plants collected from six bioregions in the study area. (a) Mean site-level seed mass (g; 100 seeds) across all six bioregions based on the final data set (with small seeds removed). Site means are shown as filled circles while bioregion means (average of all sites within a bioregion) are shown as unfilled circles. (b) Estimated mean seed mass (± 1 SE) for each bioregion based on linear mixed model analysis (see methods) of final data set (square root transformed). Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, See Fig. 2.1 for full names

Figure 5.3 Scatterplots depicting linear relationships between seed mass and tw predictor variables: (a) site-level scores on PC1 (primarily related to climate) unstandardised regression coefficient.	. B =
Figure 5.4 Scatterplots depicting linear relationships between seed mass and tw predictor variables: (b) site longitude, and (c) site latitude. $B = \text{unstandar}$ regression coefficient.	rdised
Figure 5.5 Variance in seed mass for all study sites in each bioregion, determine the variance in seed mass among seed-producing plants using the final data. Abbreviations denote the individual bioregions, See Fig. 2.1 for full names	a set.

List of Tables

Table 1.1 Examples of evidence of clinal variation in native and invasive species in response to climate throughout different life stages. 4
Table 2.1 Bioregion profiles for bioregions used in this study. Data were obtained from the NSW Department of Environment and Heritage (www.environment.nsw.gov.au; 2012) and the SILO enhanced meterological dataset and datadrill procedure (see 2.2.5 for details). Abbreviations: Pvar = annual precipitation variability; Pav = mean annual precipitation; Tav = mean annual temperature.
Table 2.2 Environmental characteristics of the six IBRA bioregions sampled within the study region during the time period May to October 1910 – 2010 and soil variables (pH, EC, %N and %C) were collected during 2010. Means are provided for based on site-level data in each bioregion. Abbreviations denote the individual bioregions, See Fig. 2.1 for full names. Tmax = maximum temperature; Tmin = minimum temperature; Tav = average temperature; FAO56 = potential evapotranspiration; Atmospheric water balance (AWB) = Rainfall – FAO56 (Rasmusson 1968); EC = electrical conductivity. Data were obtained for all study sites using GPS locations and the SILO enhanced meteorological dataset and datadrill procedure (see section 2.2.5 for details)
Table 2.3 Principal component loadings (Varimax rotation) from ten variables measured between May to October 1910-2010. Data were obtained for all study sites using GPS locations and the SILO enhanced meteorological dataset and datadrill procedure (see section 2.2.5 for details). Atmospheric water balance (AWB) was calculated by AWB = rainfall-evapotranspiration. Component loadings above 0.400 are shown in bold.
Table 2.4 Comparisons of germination traits among exotic plant species (invasive and non-invasive, M. Phillips unpublished data) and <i>Echium plantagineum</i> . Time to germination refers to the number of days between the simulated rainfall event and the first seed to germinate. Proportion of dormant viable seeds remaining at end of trial refers to the proportion of viable dormant seeds remaining after two days has passed with no germination
Table 3.1 Results of linear mixed models and linear regressions of growth traits61
Table 3.2 Results of linear mixed models and linear regressions of stature traits. 68
Table 3.3 Results of linear mixed model analysis of water use traits with bioregion as a fixed explanatory factor and site within bioregion as a random factor
Table 3.4 Predicted and observed trends in plants collected from arid environments compared to mesic environments. The following symbols indicate: + increasing and –

decreasing trait values with respect to aridity (a) or soil nutrient content (n). Abbreviations denote trait names see tables 3.1, 3.2 and 3.3 for full names77
Table 4.1 Results of linear mixed model analysis with reproductive traits as response variables in separate models. Bioregion and block were fixed explanatory variables and site nested within bioregion was a random explanatory variable. Light intensity and plant position were included in the model as covariates. SI_{time} represents time to first stem; FI_{time} represents time to first flower; SF_{time} represents time from first stem to first flower; SIV represents stem initiation variance and FIV represents flower initiation variance. Significant P -values are in bold with a value of $P < 0.05$ 92
Table 4.2 Results of simple linear regression analysis with reproductive traits as response variables in separate models. The explanatory variables are components from a principal component analysis with site level environmental variables; PC1 primarily represents climate factors; PC2 primarily represents edaphic factors. Significant P -values are in bold with a value of $P < 0.05$. SI_{time} represents time to first stem; FI_{time} represents time to first flower; SF_{time} represents time from first stem to first flower; SIV represents stem initiation variance and FIV represents flower initiation variance
Table 6.1 Summary of major results of the thesis with predicted and observed trends in E. plantagineum plants collected from arid environments compared to mesic environments. 136

Acronyms

 Δ Carbon isotope discrimination

AAGB Average above ground biomass

ACT Australian Capital Territory

AGB Above ground biomass

AI Aridity index (AI = P/PET)

ANCA Automated Nitrogen Carbon Analysis

AVH Australian Virtual Herbarium

AWB Atmospheric water balance (AWB = P - PET)

B Unstandardised regression coefficient

B_{leaf} Leaf biomass

BHC Broken Hill Complex bioregion

BGB Below ground biomass

C Carbon

COAST Coastal bioregion including south-east corner and Sydney basin

bioregions

EICA Evolution of increased competitive ability

FI_{time} Time to first flower in days

FIV Flower initiation variance

IBRA Interim Biogeographic Regionalisation for Australia – a frame work for

the grouping of regions by vegetation, climate and soil characteristics

L Largest leaf length

LAR Leaf area ratio

LMA Leaf mass area

LMM Linear mixed model

MDD Murray Darling depression bioregion

N Nitrogen

NPK Nitrogen, phosphorous and potassium

NSW New South Wales

P Precipitation

P_{area} Photosynthetic area

PC1 Principal component one representative of climate

PC2 Principal component two representative of edaphic factors

PET Potential evapotranspiration

RGR Relative growth rate

RIV Riverina bioregion

RSR Root:shoot ratio

SE Standard error

SEH South-eastern highlands bioregion

SF_{time} Time between stem initiation and flower initiation

SILO Climate database that contains Australian climate data from 1889 to

current, hosted by the the State of Queensland Department of

Environment and Resource Management 2012.

 SI_{time} Time to first stem in days

SIV Stem initiation variance

SLA Specific leaf area

SWS NSW south-west slopes bioregion

t Time in days

WUE Water use efficiency

W Total water use