

**Renosterveld remnants –  
Current ecological situation and restoration perspectives**

DISSERTATION ZUR ERLANGUNG DES  
DOKTORGRADES DER NATURWISSENSCHAFTEN (DR. RER. NAT.)  
DER NATURWISSENSCHAFTLICHEN FAKULTÄT III  
- BIOLOGIE UND VORKLINISCHE MEDIZIN -  
DER UNIVERSITÄT REGENSBURG



vorgelegt von

**Steffen Heelemann**

aus Jena

im Jahr 2010

Promotionsgesuch eingereicht am: 10.12.2010

Die Arbeit wurde angeleitet von: Prof. Dr. Peter Poschlod

Prüfungsausschuss

Prüfungsausschussvorsitz: Prof. Dr. Thomas Dresselhaus

1. Prüfer: Prof. Dr. Peter Poschlod
2. Prüfer: PD Dr. Christoph Reisch
3. Prüfer: Prof. Dr. Erhard Strohm
4. Ersatzprüfer: Prof. Dr. Joachim Ruther

## Abstract

West Coast Centre renosterveld in the Western Cape of South Africa is highly fragmented and threatened due to conversion by agriculture, urbanization and the effects of invasive alien species. Currently, insufficient data on ecological processes and restoration priorities of this endangered vegetation type exist. Therefore, this thesis is an attempt to close knowledge gaps, starting with a review of renosterveld research in chapter one. Integrating available research results, the study explored renosterveld at different levels. In chapter two, the ecosystem status of pristine and adjacent degraded renosterveld (abandoned agricultural field, pine plantation) was assessed via vegetation, soil and seed bank surveys, allowing the evaluation of restoration potential. In chapter three, the effect of smoke primer as a fire-surrogate was tested on soil seed banks of pristine and degraded renosterveld. In chapter four, experiments with alien species *Echium plantagineum* from different habitats were conducted in order to examine fire-dependency on germination behavior. In chapter five, experiments were set up to test restoration methods that are novel to abandoned fields in renosterveld. These restoration experiments mimicked natural re-colonization processes by sowing pioneer species (*Otholobium hirtum*) and installing bird perches to enhance seed rain of frugivorous birds. Furthermore, a pine clearing experiment and recovery from the indigenous soil seed bank were observed. The last three chapters assessed population genetic consequences of habitat fragmentation in two annual Scrophulariaceae (*Hemimeris racemosa* and *Nemesia barbata*). Study results for pristine renosterveld seed bank showed a high level of alien species, whereas abandoned fields had a depleted indigenous soil seed bank and insufficient restoration potential. In contrast, the indigenous soil seed bank of pine plantation was still viable and high restoration potential could be assumed. This was mirrored by the only successful restoration experiment (pine clearing). Despite promising tests, seeds from the other two restoration experiments were not capable to establish *in-vivo*. Although fire-surrogate is regarded a useful management tool to sustain high species number, caution is needed when alien infestation exists. Genetic analysis revealed usual genetic variation within and between populations and fragmentation regions for both species. Therefore, minor impact of fragmentation on the study species can be assumed. Concluding, nature conservation resources should be invested in habitat protection and restoration of pine plantations and not in abandoned fields that are very difficult to manipulate. Although habitat fragmentation influence on genetic variation was low in this study, it cannot be assumed that this is also valid for other species. Further genetic investigations in this fragmentation context should follow and are promising.

## Zusammenfassung

Das West Coast Centre Renosterveld ist ein mediterranes Strauchland in der Kapregion Südafrikas und wird bis heute durch Landwirtschaft, Urbanisierung und invasive Arten dezimiert. Leider liegen nur wenige Daten über die ökologischen Prozesse des stark gefährdeten Vegetationstyps vor. Diese Wissenslücken möchte die vorliegende Arbeit zu schließen helfen. Beginnend mit einer Literaturübersicht der bisherigen Forschung im ersten Kapitel, untersuchte die Arbeit verschiedene Aspekte des Renostervelds. Im zweiten Kapitel wurde der Ökosystemstatus pristiner und degradierter Flächen mit Hilfe von Boden-, Vegetations- und Samenbankanalysen evaluiert. Damit wurde es möglich das Renaturierungspotential von zwei untersuchten Ackerbrachen und einer Kieferplantage einzuschätzen. Der Effekt des Feuersurrogats Rauch auf die Bodensamenbank wurde im dritten Kapitel überprüft. Das vierte Kapitel beinhaltet Keimungsexperimente mit der invasiven Pflanzenart *Echium plantagineum*. Im fünften Kapitel wird über neue Renaturierungsmethoden auf den Ackerbrachen berichtet. Zudem wurden ein Kiefernkahlschlag und die darauffolgende Wiederbesiedlung mit indigenen Arten beobachtet. Die anschließenden Kapitel der Arbeit beschäftigten sich mit den genetischen Konsequenzen der Habitatfragmentierung für *Hemimeris racemosa* und *Nemesia barbata* (Scrophulariaceae). Die Studienergebnisse zeigten einen hohen Anteil exotischer Arten in der Samenbank des pristinen Renostervelds und ein unzulängliches Renaturierungspotential der Ackerbrachen. Die Bodensamenbank in der Kiefernplantage war hingegen durch indigene Arten geprägt und hat daher ein hohes Renaturierungspotential. Neuartige Renaturierungsexperimente waren trotz vielversprechender Vorversuche *in-vivo* erfolglos. Anders hingegen der Kahlschlag in einer Kieferplantage, denn hier konnten sich indigene Arten erfolgreich wiederansiedeln. Es stellte sich heraus, daß ein Feuersurrogat ein sehr nützliches Instrument in der Landschaftspflege des Renostervelds sein kann, um hohe Artenzahlen zu fördern. Jedoch ist Vorsicht geboten, wenn invasive Arten in der Samenbank vorhanden sind. Genetische Analysen deckten keine unübliche genetische Variation von *H. racemosa* und *N. barbata* auf, die auf eine Habitatfragmentierung zurückzuführen sein könnten. Daraus schlußfolgernd, sollten Ressourcen in den Habitatschutz und die Renaturierung von Kieferplantagen, und nicht in die kaum zu manipulierenden Ackerbrachen, investiert werden. Obwohl die Habitatfragmentierung keinen Einfluß auf die genetische Konstitution der beiden Arten zu haben scheint, muß dies nicht für andere Arten gelten. Weitere genetische Untersuchungen könnten durch die unterschiedlichen Fragmentierungsgrade sehr vielversprechend sein.

## Preface

Prior to European settlement, West Coast Centre renosterveld filled large proportions of the south-western tip of Africa, today known as Cape Floristic Region (CFR) and a biodiversity hotspot of global importance (Myers 1997, Mittermeier *et al.* 1998, Cowling and Pierce 1999, Myers *et al.* 2000, Myers 2001). The remaining renosterveld vegetation covers only ten percent of the former extent (Rebelo *et al.* 2006) and therefore, current research focus is on restoration methods (Krug 2004, Krug *et al.* 2004a, Krug and Krug 2007) and understanding of former and recent ecosystem processes (Kemper 1997, Kemper *et al.* 1999, Kemper *et al.* 2000, Donaldson *et al.* 2002, Raitt 2005, Muhl 2008, Radloff 2008).

A literature review was carried out to recapitulate previous renosterveld research and to reveal potential knowledge gaps. This was followed by an assessment of the ecosystem status in the largest remaining renosterveld fragment at Tygerberg Nature Reserve and its restoration potential of degraded counterparts. Furthermore, germination and restoration experiments were conducted. Also, the influence of smoke-primer treatment on soil seed banks and alien species *Echium plantagineum* was analysed. In addition, genetic variation in fragmented populations of *Hemimeris racemosa* and *Nemesia barbata* were studied. The thesis was accompanied with a launch of an online database for the study region using wiki-web technology.

This thesis is divided into eight chapters addressing the mentioned topics. Particular study sites and methodology are introduced in each chapter. Finally, summary and perspectives are given. Cited literature is given in the reference section at the end of the thesis.

## Table of contents

|  |           |
|--|-----------|
| Abstract.....  | ii        |
| Zusammenfassung.....   | iii       |
| Preface.....   | iv        |
| Table of contents .....  | v         |
| List of figures.....   | ix        |
| List of tables.....  | xi        |
| List of boxes.....   | xii       |
| List of abbreviations .....  | xiii      |
| Eidesstattliche Erklärung.....   | xiv       |
| <br>   |           |
| <b>1</b> <i>The endangered renosterveld in the Cape Lowlands of South Africa .....</i>   | <b>1</b>  |
| Vegetation .....   | 1         |
| Geology and soils.....   | 4         |
| Fauna and disturbance .....  | 4         |
| Past drivers.....  | 5         |
| The human factor.....  | 5         |
| The turning point .....  | 6         |
| Cape tribulations and accelerating pressure .....  | 6         |
| Fragmented renosterveld .....  | 7         |
| Fragmentation genetics.....  | 8         |
| Land abandonment.....  | 10        |
| What is renosterveld – grassland, shrubland or both?.....  | 10        |
| How to manage renosterveld?.....   | 12        |
| Where to go from here? .....   | 14        |
| Methodology background.....  | 16        |
| <br>   |           |
| <b>2</b> <i>Take it or leave it - degradation and restoration priorities of endangered West Coast<br/>Centre renosterveld.....</i> | <b>21</b> |
| Abstract.....  | 21        |
| Introduction.....  | 22        |
| Material and Methods .....   | 24        |
| <i>Study area and sites.....</i>   | 24        |
| <i>Vegetation and soil survey.....</i>   | 25        |
| <i>Soil seed bank sampling and germination .....</i>   | 25        |
| <i>Data analyses .....</i>   | 26        |
| Results .....  | 26        |
| <i>Vegetation survey .....</i>   | 26        |
| <i>Soil survey .....</i>   | 28        |

|   |           |
|---|-----------|
| <i>Soil seed bank survey</i> .....  | 29        |
| <i>Combination of soil seed bank and vegetation data</i> .....  | 31        |
| <b>Discussion</b> .....   | <b>33</b> |
| <i>Vegetation analysis</i> .....  | 33        |
| <i>Soil analysis</i> .....  | 33        |
| <i>Soil seed bank analysis</i> .....  | 34        |
| <i>Combination of soil seed bank and vegetation data</i> .....  | 35        |
| <b>Appendix 1</b> .....   | <b>36</b> |
| <b>Appendix 2</b> .....   | <b>38</b> |
| <br>  |           |
| <b>3 <i>Rise like a phoenix? Insights from smoke-primer experiments on pristine and degraded renosterveld soil seed bank.</i></b> ..... | <b>39</b> |
| <b>Abstract</b> .....   | <b>39</b> |
| <b>Introduction</b> .....   | <b>40</b> |
| <b>Material and Methods</b> .....   | <b>41</b> |
| <i>Study area and sites</i> .....   | 41        |
| <i>Soil seed bank sampling, germination experiment and data analysis</i> .....  | 41        |
| <b>Results</b> .....  | <b>42</b> |
| <b>Discussion</b> .....   | <b>44</b> |
| <br>  |           |
| <b>4 <i>Does fire promote rapid germination adaptation in <i>Echium plantagineum</i> (Patterson's Curse)?</i></b> .....                 | <b>46</b> |
| <b>Abstract</b> .....   | <b>46</b> |
| <b>Introduction</b> .....   | <b>47</b> |
| <b>Materials and Methods</b> .....  | <b>47</b> |
| <i>Study species</i> .....  | 47        |
| <i>Data collection and study-area</i> .....   | 48        |
| <i>Germination experiment and data analyses</i> .....   | 49        |
| <b>Results</b> .....  | <b>49</b> |
| <b>Discussion</b> .....   | <b>50</b> |
| <br>  |           |
| <b>5 <i>Pioneers, perches and pine clearing - promising restoration methods of degraded renosterveld habitats?</i></b> .....            | <b>52</b> |
| <b>Abstract</b> .....   | <b>52</b> |
| <b>Introduction</b> .....   | <b>53</b> |
| <b>Material and Methods</b> .....   | <b>55</b> |
| <i>Study area and sites</i> .....   | 55        |
| <i>Pioneer experiment</i> .....   | 55        |
| <i>Perch experiment</i> .....   | 57        |
| <i>Numerical analyses</i> .....   | 58        |
| <b>Results</b> .....  | <b>59</b> |
| <i>Pioneer experiment</i> .....   | 59        |
| <i>Perch experiment</i> .....   | 60        |
| <i>Pine clearing experiment</i> .....   | 62        |
| <b>Discussion</b> .....   | <b>63</b> |

|   |            |
|---|------------|
| <i>Pioneer experiment</i> .....   | 64         |
| <i>Perch experiment</i> .....   | 64         |
| <i>Pine clearing experiment</i> .....   | 66         |
| <b>Appendix</b> .....   | <b>67</b>  |
| <br>  |            |
| <b>6 <i>Hemimeris racemosa</i> populations sustain high genetic variation in the fragmented renosterveld of South Africa</b> .....  | <b>69</b>  |
| <b>Abstract</b> .....   | <b>69</b>  |
| <b>Introduction</b> .....   | <b>70</b>  |
| <b>Material and Methods</b> .....   | <b>72</b>  |
| <i>Species description</i> .....  | 72         |
| <i>Sampling procedure and landscape setup</i> .....   | 73         |
| <i>DNA isolation and AFLP analysis</i> .....  | 75         |
| <i>Statistical analysis</i> .....   | 77         |
| <b>Results</b> .....  | <b>78</b>  |
| <i>General genetic structure</i> .....  | 78         |
| <i>Impact of fragmentation degree on genetic structure</i> .....  | 82         |
| <b>Discussion</b> .....   | <b>86</b>  |
| <i>General genetic structure</i> .....  | 87         |
| <i>Impact of fragmentation degree on genetic structure</i> .....  | 88         |
| <i>Implications for conservation</i> .....  | 89         |
| <b>Conclusion</b> .....   | <b>90</b>  |
| <br>  |            |
| <b>7 <i>Does fragmentation really matter? Genetic variation within and between remnant populations of <i>Nemesia barbata</i> in the fragmented renosterveld of South Africa</i></b> ..... | <b>91</b>  |
| <b>Abstract</b> .....   | <b>91</b>  |
| <b>Introduction</b> .....   | <b>92</b>  |
| <b>Material and Methods</b> .....   | <b>93</b>  |
| <i>Species description</i> .....  | 93         |
| <i>Sampling procedure</i> .....   | 94         |
| <i>DNA isolation, AFLP and statistical analysis</i> .....   | 97         |
| <b>Results</b> .....  | <b>97</b>  |
| <i>General genetic structure</i> .....  | 97         |
| <i>Impact of fragmentation degree on genetic structure</i> .....  | 101        |
| <b>Discussion</b> .....   | <b>106</b> |
| <i>General genetic structure</i> .....  | 106        |
| <i>Impact of fragmentation degree on genetic structure</i> .....  | 106        |
| <b>Conclusion</b> .....   | <b>108</b> |
| <br>  |            |
| <b>8 <i>What determines genetic variation in populations - life history traits or degree of fragmentation?</i></b> .....  | <b>109</b> |
| <b>Abstract</b> .....   | <b>109</b> |
| <b>Introduction</b> .....   | <b>110</b> |
| <b>Material and Methods</b> .....   | <b>112</b> |
| <i>Species description and sampling procedure</i> .....   | 112        |
| <i>DNA isolation, AFLP and Statistical analysis</i> .....   | 113        |

|  |            |
|--|------------|
| <b>Results .....</b>   | <b>114</b> |
| <i>General genetic structure .....</i>                           | 114        |
| <i>Impact of fragmentation degree on genetic structure .....</i> | 117        |
| <b>Discussion.....</b>   | <b>119</b> |
| <i>General genetic structure .....</i>                           | 120        |
| <i>Impact of fragmentation degree on genetic structure .....</i> | 121        |
| <br>   |            |
| <b>9 Summary.....</b>  | <b>124</b> |
| <br>   |            |
| <b>10 Perspectives.....</b>                                      | <b>127</b> |
| <br>   |            |
| <b>Acknowledgements .....</b>                                    | <b>131</b> |
| <br>   |            |
| <b>References.....</b>   | <b>132</b> |

## List of figures

|   |     |
|---|-----|
| Fig. 1. Location of study area at the south-western tip of South Africa.....                                | 2   |
| Fig. 2. Fragmentation of lowland renosterveld. ....   | 2   |
| Fig. 3. State and transition model for renosterveld vegetation. ....  | 12  |
| Fig. 4. Map of the south-western Cape of South Africa. ....   | 24  |
| Fig. 5. DCA ordination of above-ground vegetation at Tygerberg Nature Reserve. ....                         | 27  |
| Fig. 6. DCA ordination of vegetation and soil parameters at Tygerberg Nature Reserve.....                   | 29  |
| Fig. 7. DCA ordination of soil seed bank at Tygerberg Nature Reserve.....                                   | 30  |
| Fig. 8. DCA ordination of above ground vegetation cover and seed bank data. ....                            | 32  |
| Fig. 9. Two rows of soil samples without and with smoke-primer treatment. ....                              | 41  |
| Fig. 10. DCA of vegetation and soil seed bank with and without smoke-primer treatment. ....                 | 44  |
| Fig. 11. Map of sampling date and location. ....  | 48  |
| Fig. 12. Germination percentage of <i>E. plantagineum</i> from different Mediterranean habitats .....       | 50  |
| Fig. 13. Map of the Cape lowlands, South Africa. ....   | 56  |
| Fig. 14. Artificial bird perching structure with netted seed trap. ....                                     | 57  |
| Fig. 15. Restoration site immediately after pine clearing (A) and one year later (B).....                   | 58  |
| Fig. 16. Plant cover values of <i>Otholobium hirtum</i> and <i>Poaceae</i> spp.....                         | 59  |
| Fig. 17. Germination treatments of <i>Otholobium hirtum</i> . ....  | 60  |
| Fig. 18. Number of dispersed seeds at Mooiplaas and Tygerberg. ....   | 61  |
| Fig. 19. DCA analysis of vegetation and soil seed bank (renosterveld, pine plantation, pine clearing). .... | 63  |
| Fig. 20. Habitus of <i>Hemimeris racemosa</i> .....   | 73  |
| Fig. 21. Sampling sites in different fragmentation regions of renosterveld. ....                            | 74  |
| Fig. 22. Sampling sites with fragment size and mean distance to neighbour fragment. ....                    | 75  |
| Fig. 23. Bayesian analysis of group allocation for <i>Hemimeris racemosa</i> . ....                         | 80  |
| Fig. 24. Bayesian analysis of group allocation for <i>Hemimeris racemosa</i> . ....                         | 81  |
| Fig. 25. Assignments of <i>Hemimeris racemosa</i> individuals to $K=2$ demes. ....                          | 81  |
| Fig. 26. Principal coordinates analysis of 398 <i>Hemimeris racemosa</i> individuals.....                   | 81  |
| Fig. 27. Spatial genetic structure of <i>Hemimeris racemosa</i> . ....                                      | 82  |
| Fig. 28. Nei's gene diversity and Shannon's Index of <i>Hemimeris racemosa</i> . ....                       | 82  |
| Fig. 29. Percentage of polymorphic loci of <i>Hemimeris racemosa</i> . ....                                 | 83  |
| Fig. 30. Principal coordinates analysis of <i>Hemimeris racemosa</i> in region A. ....                      | 84  |
| Fig. 31. Principal coordinates analysis of <i>Hemimeris racemosa</i> in regions B. ....                     | 85  |
| Fig. 32. Principal coordinates analysis of <i>Hemimeris racemosa</i> in regions C. ....                     | 85  |
| Fig. 33. Spatial genetic structure of <i>Hemimeris racemosa</i> in fragmentation region C. ....             | 86  |
| Fig. 34. Habitus of <i>Nemesia barbata</i> .....  | 94  |
| Fig. 35. Sampling sites in different fragmentation regions of renosterveld. ....                            | 95  |
| Fig. 36. Sampling sites with fragment size and mean distance to neighbour fragment. ....                    | 96  |
| Fig. 37. Bayesian analysis using logarithmic likelihood of group allocation. ....                           | 99  |
| Fig. 38. Bayesian analysis using logarithmic likelihood of group allocation. ....                           | 100 |
| Fig. 39. Individual assignments to $K=2$ demes. ....  | 100 |

---

|  |     |
|--|-----|
| Fig. 40. Principal coordinates analysis of 222 <i>Nemesia barbata</i> individuals. ....                | 101 |
| Fig. 41. Spatial genetic structure of <i>Nemesia barbata</i> . ....                                    | 101 |
| Fig. 42. Nei's Gene diversity and Shannon's Index of <i>Nemesia barbata</i> . ....                     | 102 |
| Fig. 43. Percentage of polymorphic loci of <i>Nemesia barbata</i> . ....                               | 102 |
| Fig. 44. Principal coordinates analysis of <i>Nemesia barbata</i> in regions A. ....                   | 104 |
| Fig. 45. Principal coordinates analysis of <i>Nemesia barbata</i> in regions B. ....                   | 104 |
| Fig. 46. Principal coordinates analysis of <i>Nemesia barbata</i> in regions C. ....                   | 105 |
| Fig. 47. Spatial genetic structure of <i>Nemesia barbata</i> in fragmentation region A. ....           | 105 |
| Fig. 48. Individual assignments to $K=2$ demes. ....   | 116 |
| Fig. 49. Nei's Gene diversity of <i>Nemesia barbata</i> and <i>Hemimeris racemosa</i> . ....           | 117 |
| Fig. 50. Shannon's Information Index of <i>Nemesia barbata</i> and <i>Hemimeris racemosa</i> . ....    | 118 |
| Fig. 51. Percentage of polymorphic loci of <i>Nemesia barbata</i> and <i>Hemimeris racemosa</i> . .... | 118 |
| Fig. 52. Renosterveld succession and management model. ....  | 127 |

## List of tables

|   |     |
|---|-----|
| Table 1. Species and seed number in vegetation and soil seed bank. ....                                       | 27  |
| Table 2. Comparison of chemical and physical soil parameters in different land use types. ....                | 28  |
| Table 3. Soil seed bank data on depth distribution, life forms and alien species. ....                        | 31  |
| Table 4. Soil seed bank data with and without smoke-primer treatment. ....                                    | 42  |
| Table 5. Species with significant increased germination response after smoke-primer treatment. ....           | 43  |
| Table 6. Germination data of <i>E. plantagineum</i> from different Mediterranean habitats. ....               | 50  |
| Table 7. Treatment regimes and codes for <i>Otholobium hirtum</i> . ....                                      | 56  |
| Table 8. Dispersal of diaspores at Mooiplaas and Tygerberg. ....  | 61  |
| Table 9. Comparison of soil parameters and vegetation in pristine and degraded renosterveld. ....             | 62  |
| Table 10. Perch effect with enhanced seed rain in abandoned agricultural fields. ....                         | 65  |
| Table 11. Sampled populations of <i>Hemimeris racemosa</i> . ....   | 76  |
| Table 12. Genetic variation within populations of <i>Hemimeris racemosa</i> . ....                            | 78  |
| Table 13. Pearson correlation coefficient of fragment size and distance with genetic variation. ....          | 79  |
| Table 14. Analysis of molecular variance of <i>Hemimeris racemosa</i> . ....                                  | 79  |
| Table 15. Bayesian model-based clustering likelihoods and model selection. ....                               | 80  |
| Table 16. Analysis of molecular variance of <i>Hemimeris racemosa</i> (regional level). ....                  | 83  |
| Table 17. Bayesian model-based clustering likelihoods and model selection. ....                               | 83  |
| Table 18. Mantel test with correlation coefficient of <i>Hemimeris racemosa</i> . ....                        | 86  |
| Table 19. Sampled populations of <i>Nemesia barbata</i> . ....  | 96  |
| Table 20. Genetic variation within populations of <i>Nemesia barbata</i> . ....                               | 97  |
| Table 21. Pearson correlation coefficient of fragment size and distance with genetic variation. ....          | 98  |
| Table 22. Results of analysis of molecular variance of <i>Nemesia barbata</i> . ....                          | 98  |
| Table 23. Bayesian model-based clustering likelihoods and model selection. ....                               | 99  |
| Table 24. Results of analysis of molecular variance of <i>Nemesia barbata</i> (regional level). ....          | 103 |
| Table 25. Bayesian model-based clustering likelihoods and model selection. ....                               | 103 |
| Table 26. Mantel test results of <i>Nemesia barbata</i> in three fragmentation regions. ....                  | 105 |
| Table 27. Characterization of study species. ....   | 112 |
| Table 28. Sampled populations of <i>Hemimeris racemosa</i> and <i>Nemesia barbata</i> . ....                  | 112 |
| Table 29. Number of fragments according to primer combination. ....   | 113 |
| Table 30. Genetic variation within populations of <i>Hemimeris racemosa</i> and <i>Nemesia barbata</i> . .... | 114 |
| Table 31. Pearson correlation coefficient of fragment size and distance with genetic variation. ....          | 115 |
| Table 32. Analysis of molecular variance of <i>Hemimeris racemosa</i> and <i>Nemesia barbata</i> . ....       | 115 |
| Table 33. Bayesian model-based clustering likelihoods and model selection. ....                               | 116 |
| Table 34. Analysis of molecular variance of <i>Hemimeris racemosa</i> and <i>Nemesia barbata</i> . ....       | 118 |
| Table 35. Bayesian model-based clustering likelihoods and model selection. ....                               | 119 |
| Table 36. Mantel test with correlation coefficient of genetic and geographical distances. ....                | 119 |
| Table 37. Ecosystem health, conservation and restoration of different renosterveld habitats. ....             | 130 |

**List of boxes**

*Box 1. Renosterveld sub-regions..... 3*

*Box 2. Historical data dealing with renosterveld vegetation..... 11*

*Box 3. Management guidelines for different renosterveld types ..... 13*

**List of abbreviations**

|        |  |
|--------|--|
| AF     | Abandoned fields                       |
| AFLP   | Amplified Fragment Length Polymorphism |
| AMOVA  | Analysis of Molecular Variance         |
| ANOVA  | Analysis of Variance                   |
| BHU    | Broad Habitat Unit                     |
| bp     | Base pair                              |
| C      | Carbon                                 |
| CEC    | Cation exchange capacity               |
| CFC    | Cape Faunal Centre                     |
| C:N    | Carbon - Nitrogen - ratio              |
| Cond.  | Conductivity                           |
| DCA    | Detrended Correspondence Analysis      |
| DNA    | Deoxyribonucleic acid                  |
| dNTP   | Desoxynukleotid-5-Triphosphat          |
| E      | East                                   |
| GIS    | Geographic Information Systems         |
| K      | Potassium                              |
| kb     | Kilo base pair                         |
| LGM    | Last Glacial Maximum                   |
| LSD    | Fisher's least significant difference  |
| LTD    | Limited                                |
| N      | Nitrogen                               |
| P      | Phosphorus                             |
| PCA    | Principal Component Analysis           |
| PCOA   | Principal Coordinates Analysis         |
| PCR    | Polymerase chain reaction              |
| PP     | Pine plantation                        |
| Pty    | Proprietary limited company            |
| RNA    | Ribonucleic acid                       |
| RSA    | Republic of South Africa               |
| RV     | Renosterveld                           |
| S      | South                                  |
| S.D.   | Standard deviation                     |
| S.E.   | Standard error                         |
| s.l.   | Above sea level                        |
| T.N.R. | Tygerberg Nature Reserve               |
| VAM    | Vesicular-arbuscular mycorrhiza        |
| WHC    | Water holding capacity                 |
| y      | Year                                   |
| ybp    | Years before present                   |
| ~      | Mean                                   |
| '      | Minutes                                |

## **Eidesstattliche Erklärung**

Ich erkläre hiermit an Eides statt, daß ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

Bei der Auswahl und Auswertung folgenden Materials haben mir die nachstehend aufgeführten Personen in der jeweils beschriebenen Weise unentgeltlich geholfen:

1. Prof. Dr. Peter Poschlod (Universität Regensburg): Themenauswahl, inhaltliche Besprechung der Arbeit und Auswertung der erhobenen Daten
2. PD Dr. Christoph Reisch (Universität Regensburg): Themenauswahl, inhaltliche Besprechung der Arbeit und Auswertung der erhobenen Daten
3. Prof . Dr. Karen J. Esler (Universität Stellenbosch): Themenauswahl, inhaltliche Besprechung der Arbeit
4. Dr. Cornelia Krug (Universität Kapstadt): Themenauswahl, inhaltliche Besprechung der Arbeit
5. Dr. Gyan Sharma (Universität Varanasi): Inhaltliche Besprechung des 4. Kapitels

Weitere Personen waren an der inhaltlich-materiellen Herstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich hierfür nicht die entgeltliche Hilfe eines Promotionsberaters oder anderer Personen in Anspruch genommen. Niemand hat von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen. Die Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Regensburg, 10.12.2010

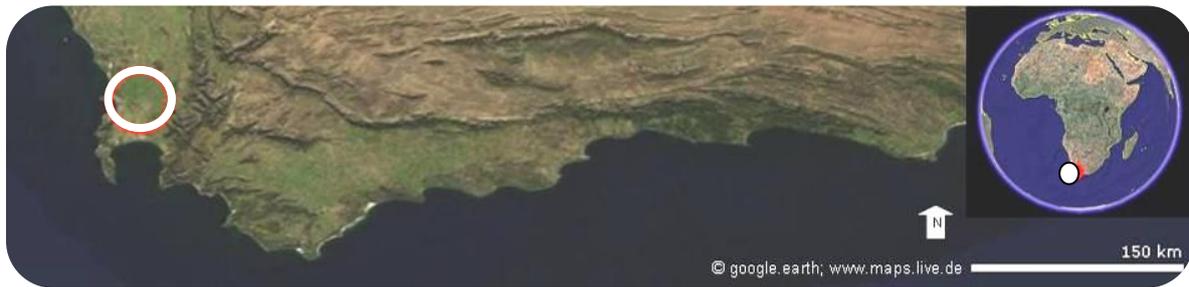
Steffen Heelemann

# 1 The endangered renosterveld in the Cape Lowlands of South Africa

## Vegetation

Renosterveld is a Mediterranean-type shrubland (*sensu* Di Castri and Mooney 1973, Specht and Moll 1983) in the South-west corner of Africa (Fig. 1). The vegetation is evergreen, fire-prone, cupressoid-leaved, dense and mid-high (Rebelo *et al.* 2006) with clumps of tall, ornithochorus and broad-leaved thicket species or grasses that occur on ancient termite mounds (heuweltjies) formed during the Pleistocene (Krug and Krug 2007). Renosterveld is characterized by members of the Asteraceae family (*Eriocephalus*, *Helichrysum*, *Oedera*, *Pteronia* and *Relhania*) and dominated by the Renosterbos (*Elytropappus rhinocerotis*) with a grassy understorey and high diversity of geophytes (Boucher 1980, Moll *et al.* 1984, McDowell and Moll 1992). Other subdominant shrub families are Boraginaceae, Fabaceae, Malvaceae, Rosaceae and Rubiaceae (Goldblatt and Manning 2000). Geophytes derive from both, monocots (Amaryllidaceae, Asparagaceae, Iridaceae, Hyacinthaceae, Orchidaceae) and dicots (Oxalidaceae, Geraniaceae), and many species are now economically important cut flowers, such as *Freesia*, *Ixia*, *Ornithogalum* and *Pelargonium* (Rebelo *et al.* 2006). Prior to anthropogenic transformation, renosterveld filled large proportions (Fig. 2) of the Fynbos Biome (Kruger 1984), which coincides with the Cape Floristic Region (CFR) and the Cape Floral Kingdom, the smallest of the world's six floral kingdoms (Takhtajan 1969, Good 1974). It is one of the world's most species-rich region harbouring 8.971 flowering plant species with 68 percent of them being endemic (Goldblatt and Manning 2000, Goldblatt *et al.* 2008). The Cape region supports much higher species numbers per area compared to other Mediterranean-type ecosystems (Cowling *et al.* 1996). The high species numbers result from accumulation of many lineages over the entire Cenozoic (Linder 2006) and high beta and gamma species turnover that had lead to the highest plant diversity in the outer-tropics (Cowling *et al.* 1992). Models suggest that either abiotic (Cowling *et al.* 1992, Goldblatt 1997, Cowling *et al.* 2009) or biotic factors (Johnson 1996) are responsible for these speciation processes. In the entire ecoregion, 1.435 plants and 112 animals are listed as red data book species, most of them highly endangered and threatened, especially in the lowlands (Rebelo 1992a, Rebelo *et al.* 2006). The region is regarded a biodiversity hotspot by Conservation International (Myers *et al.* 2000) and a centre of plant diversity by WWF-IUCN (Davis *et al.* 1994). Cowling and Heijnis (2001) categorized renosterveld as one of eight

Broad Habitat Units (BHUs). Here, approximately 800 taxa occur of which only a few are endemic (Wood and Low 1993a, b).



**Fig. 1. Location of study area at the south-western tip of South Africa.**  
Dot and circle indicate study area at the Cape lowlands with nearby Cape Town.

Similar to other Mediterranean biomes (Underwood *et al.* 2009), the Cape lowlands and renosterveld in particular were heavily transformed due to agriculture (Fig. 2), urbanisation and alien plant invasion (Cowling and Pierce 1999, Heijnis *et al.* 1999, Kemper *et al.* 1999). However, conservation status is very poor and reservation targets have not been achieved, as only 0.6 percent of renosterveld is under protection (Cowling *et al.* 1999a). This is despite global biodiversity importance (Cowling and Pierce 1999) and regional conservation priority (Rebello 1997).



**Fig. 2. Fragmentation of lowland renosterveld.**

Extent of renosterveld (map) prior transformation (grey areas) with remaining renosterveld remnants (dark areas). Data based on von Hase *et al.* (2003b). Recent agriculture (picture) on former renosterveld with original vegetation occurring on hills only (horizon, right side).

The vernacular term renosterveld - meaning “rhinoceros veld” in Afrikaans - derived either from historical sightings of the black rhinoceros (*Diceros bicornis*) in this vegetation type or from “renosterbos veld” that refers to the dominant asteraceous shrub *Elytropappus rhinocerotis* (Boucher 1980). Black rhinoceros were the only animals feeding on phenol-rich *E. rhinocerotis*, which is one explanation for the name (Rebello *et al.* 2006). Furthermore, the dark and greyish appearance of the vegetation could have the origin for the term Swartland,

meaning black field. West Coast Centre renosterveld is known for high geophyte diversity (Iridaceae, Liliaceae, Orchidaceae and Oxalidaceae) and winter growing (C<sub>3</sub>) grasses. In contrast South Coast Centre renosterveld has a higher abundance of summer growing (C<sub>4</sub>) grasses and lower geophyte diversity. Namely, four biogeographically defined renosterveld sub-regions (Box 1) are distinguished (Moll *et al.* 1984, Rebelo *et al.* 2006).

- **Mountain Centre**
  - Nieuwoudtville to Oudtshoorn, east of Cederberg, north of Langeberg
  - Xeric inland renosterveld, lower cover (25-60 %) depending on moisture
  - Dominant species are *Elytropappus rhinocerotis*, *Relhania genistifolia* and *Pteronia incana*. Higher proportion of succulents occur
  - Mainly C<sub>4</sub> grasses occur, but reduced to overgrazing
  - *Acacia karroo*, *Aloe ferox* and *Euclea undulata* are scattered elements
- **Eastern Centre**
  - Uniform *Elytropappus rhinocerotis* dominated shrubland
  - Mainly C<sub>4</sub> grasses occur, but reduced to overgrazing
  - Strong affinities to Albany Thicket and grasslands
- **South Coast Centre**
  - South of Langeberg and Riviersonderend Mountains
  - Less geophytes and higher proportion of C<sub>4</sub> grasses
  - Dominant species include *Elytropappus rhinocerotis* with subdominant *Oedera*, *Helichrysum* and *Hermannia* species, cover 50-75 %
- **West Coast Centre**
  - West of Hottentots Holland and Twenty-four River Mountains
  - Sparser grass cover with C<sub>3</sub> grasses, such as the genera *Erharta*, *Pentaschistis*, *Merxmuellera*, *Tribolium*, *Cymbopogon* and *Themeda*
  - Higher proportion of deciduous geophytes and annuals
  - High cover 50-90 % with dominant *Elytropappus rhinocerotis* and subdominant *Eriocephalus africanus* and *Leysera gnaphaloides*
  - Heuweltjies support thicket elements, e.g. *Olea europaea ssp. africana*

**Box 1. Renosterveld sub-regions.**

Extracted from Moll *et al.* 1984, Rebelo *et al.* 2006.

## Climate

Renosterveld is characterized by typical Mediterranean climate (summer drought and winter rain with 80 % of precipitation received between April and September) and grows in areas with rain fall between 250 and 600 mm per year depending on altitude (Cowling *et al.* 1997b). Drier than these parameters it develops into Succulent Karoo, wetter than this asteraceous fynbos is found. Temperatures in the region are mild and it is virtually frost free. Summer temperatures reach a maximum of 30 °C. The cold Benguela current is responsible for stable

climatic conditions and is also a source of fog. The region is wind affected and seasonal wind patterns have a cooling effect on temperatures (Lambrechts 1998). High wind speeds occur during summer with the prevailing trade winds from the south-east (Deacon *et al.* 1992). A higher proportion of winter rainfall occurs in the west of the Cape region, whereas rainfall season in the south-east is less pronounced, which has profound implications for the ecology of species occurring on this gradient (Heelemann *et al.* 2008, Heelemann *et al.* 2010). The following paragraphs will further characterize West Coast Centre renosterveld and put it in relation to general information provided about renosterveld.

### **Geology and soils**

West Coast Centre renosterveld occupies an area of 6.141 km<sup>2</sup> in the coastal foreland between Piketberg and Somerset West. It occurs on fine-grained clay and silt soils derived from Devonian-Ordovician sediments (Bokkeveld Group) and underlying Precambrian sediments of the Malmesbury Group (Deacon *et al.* 1992). These ancient sediments were exposed with the breakup of Gondwanaland and folded in the next 50 million years (Cowling 2001). After that, geology remained relatively stable. Ferralitic (highly weathered) and siallitic soils are present and especially the latter soils are rich in clay minerals such as illite and vermiculite (Lambrechts 1998). Malmesbury shales are now forming most parts (86 %) of the undulating plains of the West Coast Centre with some outcrops of cape granite (Deacon *et al.* 1992, Rebelo *et al.* 2006). Granite and silcrete soils are found to a lesser extent, 6 % and 3 %, respectively. Depending on substrate three vegetation types are found in the region, namely Swartland shale renosterveld, Swartland granite renosterveld and Swartland silcrete renosterveld.

### **Fauna and disturbance**

The Cape region is also home to a distinct invertebrate (Struckenberg 1962) and vertebrate fauna (Branch 1988, Crowe 1990, Skelton *et al.* 1995, Cowling and Pierce 1999). Within the Cape Faunal Centre (CFC) around 300 bird and 100 mammal species have been recorded (Cowling 2001). West Coast Centre renosterveld is a particularly important ecosystem for the Geometric Tortoise *Psammobates geometricus* endemic to the region, and one of the rarest tortoise species worldwide (Baard 1993). The fertile soils of West Coast Centre lowland renosterveld supported large herds of herbivores, e.g. African bush elephant (*Loxodonta africana*), black rhinoceros (*Diceros bicornis*) and many antelope species. In turn they attracted predators, such as lion (*Panthera leo*) and cheetah (*Acinonyx jubatus*). Renosterveld

endemic species like the blue antelope (*Hippotragus leucophaeus*) were hunted to extinction in the 18<sup>th</sup> century (Krug *et al.* 2004b, Krug and Krug 2007); the Quaqqa (*Equus quagga quaqqa*) became extinct in the early 1900s. However, populations of the endemic Bontebok (*Damaliscus pygargus*) still occur in nature conservation areas. It is suggested that game occurring in the area played a major role in the former disturbance regime. Additionally, porcupines are important ecosystem engineers (Bragg *et al.* 2005). Regular fires occurring 3-10 (up to 40) years have a large impact on vegetation (Rebello 1992b, Rebello *et al.* 2006). The disturbance magnitude, its influence, as well as origin of renosterveld are still subject to discussions (Cowling *et al.* 1986, Rebello 1992b, Newton and Knight 2004, Radloff 2008).

## Past drivers

The understanding of ecological processes in pristine renosterveld is very limited (Rebello 1995, Krug 2004, Krug *et al.* 2004a, Rebello *et al.* 2006). Newton and Knight (2004) postulate that during the Last Glacial Maximum (20.000 ybp), average temperatures were 5 °C lower and sea level 140 m deeper than today. Grasslands with large herbivores were common in the region. In the following 10.000 years, temperatures and sea levels started to rise and today's climatic belt has established. This resulted in grasslands and shrublands moving southwards. 10.000 ybp, the recent interglacial started with drier conditions and grasslands became isolated. Archaeological evidences from that time show a major anthropogenic-induced reduction and extinction of large herbivores. At 5.000 ybp, asteraceous shrubs were replacing grasslands due to summer droughts, which accelerated the large herbivore extinction. Increasing precipitation but remaining summer drought around 4000 ybp caused that only C<sub>3</sub> grasses were able to survive, which was promoted by regular burns. This shrub-grass matrix was able to carry large herbivore populations (Skead 1980). During this period nomadic hunter-gathers inhabited the region and had little impact, such as geophyte digging and small-area burns to increase their abundance as well to attract herbivores with these fresh pastures (Deacon *et al.* 1992).

## The human factor

Archaeological evidence, such as axes from the Earlier Stone Age suggest human occupation of the region beginning 1.8 million years ago (Humphrey 1998). In the Late Stone Age (20.000 ybp), nomadic hunter-gatherers (San) appeared and altered the environment only with small-scale fires to attract game (Wood and Low 1998). At around 2000 ybp, pastoralists from Botswana - also known as KhoiKhoi - arrived in the region. On their way south, they

acquired herds of goats, sheep and cattle (Schweitzer and Scott 1973, Klein 1986). The impact of those herds on the natural vegetation (Wood and Low 1998) and their seasonal movement is unknown (Sealy and Yates 1994, Balasse *et al.* 2002), but it is suggested they have seasonally followed sufficient pasture grounds (Humphrey 1998). However, the KhoiKhoi used short fire cycle in order to enhance grass growth for the intensive grazing of herds and returned 1-4 years later (Thom 1952, 1954). In general, hunting pressure was low and large areas remained undisturbed (Klein 1974, Krug and Krug 2007).

### **The turning point**

A significant change in land use practices occurred after the arrival of Europeans in 1652 (Newton 2008). Jan van Riebeeck established a station for the Dutch East Indian Company in Cape Town and Europeans survived by trading food with the KhoiKhoi. However, the KhoiKhoi were no longer willing to trade in the following years and so European expansion began (Wesson 1998). From 1679 onwards, Simon van der Stel commanded to intensify the conversion into agricultural land for cereal crops (northern lowlands, today's Swartland), vineyard (southern lowlands, today's Winelands) and cattle. His aim was to develop a self-sufficient colony and he succeeded to the point of overproduction by giving away freehold land and seed. Livestock was held stationary and shorter fire cycles were applied to promote sufficient pastures. However, fire suppression was practised from 1678 onwards to protect certain agricultural areas and properties (Newton 2008). Hunting pressure on indigenous game was increasing significantly and large herbivores were extinct by the end of the 19<sup>th</sup> century (Newton 2008).

### **Cape tribulations and accelerating pressure**

In the 19<sup>th</sup> and 20<sup>th</sup> century, the Cape region suffered from several wars, economic depressions, and outbreaks of animal and plant diseases. As a result, agriculture went through several changes favouring either wheat, grape or wool products. The discovery of gold in 1886 and diamonds in 1896 have lead to significant population growth and development of the Cape region, which resulted in more infrastructure and intense agriculture to meet human needs. A major extension of the existing agricultural land followed the onset of the First World War in 1917, with the result of only small remnants of natural renosterveld vegetation remaining. All arable land was devoted to grain production under governmental policy (Talbot 1947). From the early 20<sup>th</sup> century, farmers forced the Government to enact several protectionist policies on wheat and wine (Talbot 1947, Toerien 2000). This trend accelerated

with the rise of the National Party in 1948, leading to non-economic expansion of agriculture, soil erosion (Delius and Schirmer 2000, Meadows 2003) and preference of white Afrikaans-speaking citizens (Wilson 1971, Wesson 1998). This situation left the country in a socio-economic crisis, ending only with the introduction of a democratic system in 1994 (Dietrich *et al.* 2004). By the 1950s, soils were heavily degraded, especially in the Swartland and on steeper slopes (Newton 2008). This degradation could only be stopped due to policy changes in the 1980s (Newton and Knight 2005). Within this process, agricultural land was abandoned and remaining renosterveld fragments were treated like crop fields with use of fertilizer, herbicide and pesticide (Donaldson *et al.* 2002). Today, major threats for West Coast Centre renosterveld are further habitat loss and fragmentation (Bond *et al.* 1988, Cowling and Bond 1991, Kemper *et al.* 1999), agriculture (Boucher 1981, Hall 1981, Rebelo 1992b), urbanisation (Wood *et al.* 1994), growing socio-economic pressure (McDowell and Moll 1992) and alien plant species (Musil *et al.* 2005). Introduced Mediterranean grasses (e.g. *Avena*, *Briza*, *Lolium*) are common and invasive, being focus of recent research (Krug and Krug 2007). Also, viticulture and new agricultural activities, such as olive farming, increase in the region and threaten renosterveld (Rouget *et al.* 2003, Dietrich *et al.* 2004, Fairbanks *et al.* 2004). Moreover, climate change is most likely to have a negative impact and will increase extinction risk of local endemic species (Rutherford *et al.* 1999). The first systematic survey in renosterveld, aimed to identify remnants of conservation priority, was carried out by Jarman (1986). According to Rebelo *et al.* (2006) Renosterveld extents 27962 km<sup>2</sup> of which 80 % are transformed. The situation is even more severe in West Coast Centre renosterveld where only 10 % are left in 1.175 isolated remnants with the majority smaller than 5 ha (von Hase *et al.* 2003b, Rebelo *et al.* 2006). Those remaining fragments are 100 % irreplaceable to meet conservation targets (Cowling and Heijnis 2001, von Hase *et al.* 2003b).

### **Fragmented renosterveld**

Habitat loss and fragmentation are globally accelerating and induces size reduction and isolation of populations, as well as decreasing habitat quality and biotic interactions (Saunders *et al.* 1991). For renosterveld, negative impacts of fragmentation on pollinators (e.g. movement, abundance and composition) have been demonstrated, which in turn influenced pollination-limited plant species and their reproductive success (Donaldson *et al.* 2002, Pauw 2004, Vrdoljak and Samways 2005). Furthermore, fragmentation negatively affects seed dispersal processes (Kemper *et al.* 1999, Shiponeni 2003). However, even small fragments are able to carry viable pollinator and plant populations within an agriculture dominated matrix

(Cowling and Bond 1991, Kemper *et al.* 1999). Nevertheless, it will be necessary to buffer and enlarge fragments by restoring adjacent agricultural and abandoned land, vineyards and other degraded habitats (Rebelo 1995). This is necessary to meet UN Convention conservation goals, and to protect them from further habitat transformation and loss, as well to prepare for global change impact. Any restoration attempts need to acknowledge the past and present ecological drivers of the ecosystem to insure efficiency and sustainability of the restoration aims and efforts (Krug 2004, Krug and Krug 2007). In the past, herbivores and fire were the main ecological drivers in the renosterveld ecosystem (Radloff 2008). Herbivores are dispersal and disturbance vectors, thereby reducing grass cover and supporting shrub establishment (Midoko-Iponga 2004, Midoko-Iponga *et al.* 2005). Fire is contrasting this effect by reducing shrub cover and enhancing grass and geophyte abundance (Cowling *et al.* 1986, Manning *et al.* 2002, Proches *et al.* 2006). Nowadays, influence of herbivores and fire is altered. As a consequence, long-distance dispersal of endozoochorous plant species is reduced (Shiponeni 2003, Shiponeni and Milton 2006) and with increased vegetation cover and less gaps, establishment of shade-tolerant and long-lived shrubs became possible (Boucher 1983). General fire suppression and sequential heavy overgrazing after burning resulted in mono-specific stands of *Elytropappus rhinocerotis*, an unpalatable, anemochorous and long-lived shrub (Krug and Krug 2007). Renosterveld remnants are highly impacted by surrounding agricultural activities, such as, fertilizer and pesticide use, grazing ground, oversowing and fire (Donaldson *et al.* 2002). Especially, nutrient enrichment coupled with overgrazing and frequent fires favours alien grass and invasion processes (van Rooyen 2004, Muhl 2008). Furthermore, invasion of alien pine species is also a massive problem (Rouget *et al.* 2003). However, these treatments are low compared to irreversible impacts, such as ploughing, mining and urban development (McDowell and Moll 1992, McDowell 1995).

### **Fragmentation genetics**

Fragmentation is regarded as a particular form of anthropogenic induced environmental degradation with species-specific effects on genetic variation (Young *et al.* 1996, Haila 2002). In general, dimension of genetic variation depends on plant functional traits (Hamrick and Godt 1996, Nybom and Bartish 2000). Because plant populations are spatially structured, landscape genetics have become an important research field (Manel and Segelbacher 2009). Molecular techniques, such as Amplified Fragment Length Polymorphism (AFLP), enable the analysis of genetic variation in species and furthermore allow setting conservation goals with the aim to maintain genetic variation within and between fragmented populations. This will

help to avoid inbreeding depressions (Saccheri *et al.* 1998), ensure evolutionary processes (Lande and Barrowclough 1987) and enhance species persistence in a global change scenario (Hedrick and Miller 1992). The anthropogenic land use resulted in converted habitats and fragmented plant populations, thereby reducing population sizes and increasing population distances. The critical patch size to sustain viable populations depends on several factors, such as population genetics of the species (Fahrig 2001). Many studies were carried out evaluating the relation between population size and genetic variation (Oostermeijer *et al.* 2003). Generally speaking, correlation between population size and genetic variation is positive. Self-compatible, rare plants and a short life span will result in less genetic variation compared to outcrossing, common and long-lived species (Hamrick *et al.* 1979, Leimu and Mutikainen 2005). However, the need to incorporate population isolation as a parameter to evaluate spatial genetic structure is pointed out (Ouborg *et al.* 2006). Gene flow pattern in naturally fragmented plant populations are well studied (Larson *et al.* 1984, Ellstrand and Marshall 1985) and such species are relatively prone to inbreeding depression (Huenneke *et al.* 1991). However, little is known about these processes in anthropogenic-caused fragmentation (Lacy 1987, Lande and Barrowclough 1987, Robinson and Quinn 1992, Montalvo *et al.* 1997), where viability of remnant populations can be uncertain and critical (Tansley 1988). This is especially true for common species that are less prone to habitat fragmentation effects (Bijlsma and Loeschcke 2005). A biosphere framework for the lowlands (Heijnis *et al.* 1999) is an important option in order to prevent further habitat loss and to enhance connectivity and gene flow between renosterveld remnants. Not only biodiversity pattern should be considered in conservation, the underlying processes are equally important and should be considered (Cowling and Pressey 2001). As molecular systematic can help setting conservation goals (Soltis and Gitzendanner 1999) the same applies for population genetics. Rather than single approaches, managers should ask for full array of conservation resources including genetic analysis in order to try an integrated conservation approach (Falk 1990). *In-situ* conservation approaches are preferred to preserve genetic and ecological information (Hamilton 1994, Watson-Jones *et al.* 2006). General pollinators should not be overlooked to ensure sufficient gene flow (de Merxem *et al.* 2009). In contrast to the broad pollinator spectrum in Europe (Kwak and Bekker 2006), they are more specific in South Africa (Johnson 1996, Johnson and Steiner 2000, Donaldson *et al.* 2002, Pauw 2007), which demands consideration in any research or management initiative.

## **Land abandonment**

Land use change compromises abandonment of agricultural land (Houghton 1991, Hobbs and Cramer 2007b). Such areas are termed abandoned fields and helped to develop concepts of ecological succession (Hobbs and Walker 2007). Land abandonment is characteristic for the anthropogenic-ecosystematic relationship and environmental and socio-economic changes increase these areas worldwide (Ramankutty and Foley 1999, Cramer *et al.* 2008). Although derived from destruction of pristine habitats, abandoned fields are common and important ecosystems and interesting in many ways, for example as restoration and conservation sites, carbon sink and from the socio-economic point of view. Hence, they need a better understanding (Hobbs and Cramer 2007a). Restoration goals need to be based on the ecological reality and socio-economic setting (Hobbs and Cramer 2007a). Agricultural systems are a complex of socio-ecological relationships and therefore reasons for land abandonment (e.g. ecological, economical and socio-political changes) differ regionally (Hobbs and Cramer 2007b). Once an agricultural field is abandoned, it will follow succession and abiotic and biotic stress influences the development after abandonment (Ewel 1999). This can be positive or negative depending on human perspective and ecosystem constrains on anthropogenic management for their persistence (Hobbs and Cramer 2007b). Most of these abandoned fields are characterized by dramatically changed ecosystem processes and species composition. Therefore, they are regarded novel or emerging ecosystems and establishment of pre-existing vegetation seem not to be an appropriate restoration goal (Hobbs *et al.* 2006).

## **What is renosterveld – grassland, shrubland or both?**

The base for vegetation science in South Africa are Acock's Veld types, which are surrogates for similar farming potential of an area and coincide with vegetation types (Acocks 1953). Renosterveld vegetation occurs on granite, shale or silcrete substrates (Boucher and Moll 1981) between mountain fynbos and strandveld vegetation (Trollope 1970). It was also named cape transitional small-leafed shrubland (Cowling 1984), indicating that renosterveld is indeed very heterogeneous and difficult to describe ecologically (Boucher 1980, Boucher and Moll 1981, Boucher 1983, 1987, Newton and Knight 2004). Newton and Knight (2004) suggested that renosterveld is the result of suboptimal agricultural practice and an inappropriate term from the etymological point of view, because it rather refers to a different vegetation type than one dominated by "Renosterbos" (*Elytropappus rhinocerotis*, Asteraceae). The authors analysed historical records (Box 2) and postulated that West Coast

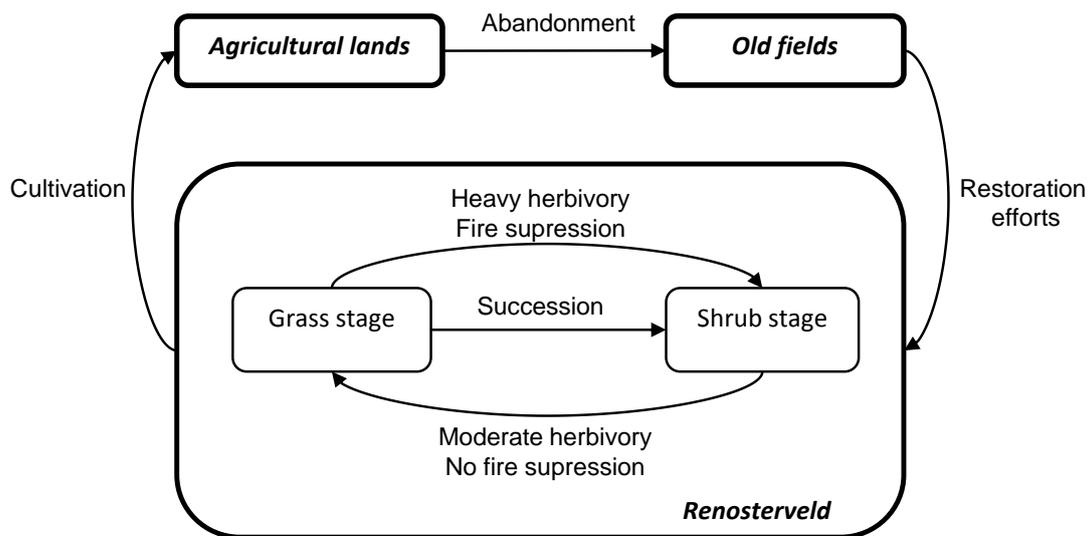
Centre renosterveld was a grassland over the last half million years with open shrubland character during the short interglacials.

- In the late 1600's up to forty wagons of grass were collected each year at Tygerberg (Theal 1922).
- In 1611, Alderworth was impressed by the lush grass vegetation and number of cows in the region (Newton and Knight 2004).
- Van Riebeeck describes in his journal the rich pastures and amount of cattle in the Cape lowlands (Thom 1952).
- Historical records indicate that *E. rhinocerotis* abruptly dominated renosterveld with hay production declining rapidly in the early 17<sup>th</sup> century (Rebelo 1996).
- In 1685 Simon van der Stel described their camp area covered with "*Rhenosters bosch*" (Waterhouse 1932). However, the camp site was set up in mountain fynbos vegetation.
- Valentyn (1726) stated that "no lovelier grass fields" were found in the Botteleray area than in the rest of Africa.
- Burchell (1811) states the term "*Rhinoster bosch*" refers to several *Stoebe* species. Also, areas still covered with renosterveld are mentioned historically for their grassland character.

**Box 2. Historical data dealing with renosterveld vegetation.**

Compiled from Newton and Knight (2004).

Krug and Krug (2007) summarized that European settlement, subsequent fire suppression and large herbivore extinction have led to a breakdown of ecosystem processes, resulting in the dominance of *E. rhinocerotis*. From that background, the authors developed a state and transition model with fire and herbivory being the main drivers of renosterveld system in the past (Fig. 3). Today, both factors function inappropriately, leading to senescence vegetation and therefore introduction of fire and large herbivores into the system is imperative (Radloff 2008). This situation makes it difficult to define a general management aim for renosterveld, which is currently a shrubland (Rebelo *et al.* 2006). Also, only 10 % of the former renosterveld extent is still viable and therefore it is unlikely that natural ecosystem processes persist in the future (Rebelo *et al.* 2006).



**Fig. 3. State and transition model for renosterveld vegetation.**  
Adapted from Krug and Krug (2007).

### How to manage renosterveld?

The recent degree of biosphere degradation is unparalleled (Ehrlich and Ehrlich 1981). Although conservation and restoration biology will not be able to return ecosystem to its prior state, it can help to slow down destruction rate (Soulé 1985). To reach conservation goals, renosterveld fragments have 100 % irreplaceable status (Cowling and Heijnis 2001, von Hase *et al.* 2003b). Although fine scale conservation plans exist, they lack implementation (Holmes and Richardson 1999). Nevertheless, basic management guidelines exist for particular types of renosterveld (Box 3). It is also important to incorporate socio-economic aspects into conservation attempts in order to provide income for underprivileged community members via ecotourism and game farming (Rebelo 1995). Succession processes are the underlying principles of habitat restoration and therefore such attempts will try to overcome factors that inhibit ecosystem development (Bradshaw 1987). Those restricting factors in abandoned fields of West Coast Centre renosterveld of South Africa (Krug and Krug 2007) seem to be similarly high in Australia (Standish *et al.* 2007) and California (Eliason and Allen 1997). Furthermore, knowledge about ecology of pristine renosterveld and its management are very limited (Rebelo 1995). Therefore, defining conservation aims and management practices is difficult (Wood and Low 1993b). Furthermore, when restoration recommendations were given, only few studies showed positive results (Wood and Low 1993b, Krug and Krug 2007). Renosterveld restoration research focused on succession of abandoned fields, for example near Elandsberg Nature Reserve. These degraded areas

developed a similar vegetation structure like surrounding pristine vegetation, but encountered less species richness and diversity compared to pristine vegetation (Krug *et al.* 2004a, Walton 2006). Especially understorey species were not able to re-establish. This could be the result of dispersal and establishment limitations, which is very common in fragmented and degraded landscapes (Bakker *et al.* 1996, Bakker and Berendse 1999). Such pattern may also exist in renosterveld where wind and herbivores are the main dispersal vectors and seed density is closely related to seed source distance (Krug 2008). Mainly alien grasses build the anemochorous and endozoochorous seed rain (Shiponeni 2003).

**General guidelines** (CapeNature 2000, Holmes *et al.* 2008)

- Control alien grasses
  - Maintaining cover of indigenous plant species
- Erade alien grasses
  - Mowing and removal to offset costs, repeat every year to deplete soil seed bank
  - Herbicide application coupled with late summer burn or heavy seasonal grazing
- Gerbil control
  - Installation of traps or perches to attract raptors
- Management
  - Using light, seasonal spring grazing coupled with autumn fire
  - Allowing grazing but prevent overgrazing and leaving sections unploughed
  - Using fertilizers and poison correctly
- Conservation
  - Identifying sensitive areas, rare plants and treats to ensure species survival
  - Enhancing conservation status (e.g. proclaiming private nature reserves)

**Palatable renosterveld** (with some alien invasion)

- Apply temporal fluctuating grazing regime at low intensity one year after fire
  - Focus on reproduction of palatable species
  - Regime should control alien grass invasion (Boucher 1995)
- Mow grass-infested renosterveld prior seed set
  - Fed grass biomass to offset costs (Musil *et al.* 2005)

**Unpalatable renosterveld** (over-grazed shrubland with dominant renosterbos)

- Rest, periodic fires, grazing, re-introduction of palatable species (Boucher 1995)
- Autumn fire (4-6 y), light spring grazing, species introduction (Holmes 2008)

**Degraded renosterveld** (cultivated fields, abandoned fields)

- Maintain status to reach certain biodiversity goals
  - Flower display, rare plant habitat (Boucher 1995)
- Apply gypsum to reduce phosphorus levels and ripping treatment to reduce soil compaction (Holmes *et al.* 2008)

**Box 3. Management guidelines for different renosterveld types**

Compiled from different sources.

Very few indigenous plant species are observed in these abandoned fields because establishment is prevented by the dominating alien grass vegetation. It is evident that grass competition had a stronger effect on establishment than herbivory (Midoko-Iponga 2004, Midoko-Iponga *et al.* 2005). This fact, coupled with the negative effect of herbivory (i.e. seed predation, seedling removal, trampling), diminishes establishment of species (Davies 1985, Bonser and Reader 1995). Management guidelines for degraded types of renosterveld are shown in Box 3. Firstly, abandoned fields should be left untouched with uncertain outcome or used for alternative purposes (Boucher 1995). Secondly, burning should be prohibited and grazing allowed. Depending on grazing intensity, this could favour palatable or unpalatable shrubs species. Thirdly, brush-cutting could favour hemi-cryptophytes and geophytes. However, experiments on these management regimes are still missing. The current restoration methodology aims to reduce alien grass cover (Krug 2004, Krug *et al.* 2004a) and is oriented towards protocols for fynbos restoration (CapeNature 2006) but facing similar problems and low success rate. Combined brush cutting, burning and herbicide application also proved to be an ineffective method to reduce grass abundance (Midoko-Iponga 2004, Midoko-Iponga *et al.* 2005). However, experiments with herbicide application coupled with light burning and additional herbicide application showed success (Musil *et al.* 2005). Both studies state that herbicide use is the most expensive method. Alternative approaches suggest mowing and removal of grass (Musil *et al.* 2005) as well as oversowing with indigenous shrub species. The latter technique is not very practical because seeds are not easy to obtain (Krug and Krug 2007). The authors concluded that abandoned fields are trapped in the alien grass state. The problem is twofold, alien grass invasion are not easy to control and they are diaspore sources for invasion processes into pristine renosterveld (Musil *et al.* 2005, Muhl 2008). Recent experiments indicate that only combined fire and herbivore pressure is able to induce a shift in community assembly (Radloff 2008) from unpalatable to palatable shrubland and *vice versa*. However, these trials had little effect on alien *Cynodon dactylon* grasslands and uncertainty prevails if these habitats will ever reach a different state via prolonged herbivore absence (Radloff 2008).

### **Where to go from here?**

In the 20<sup>th</sup> century, South African agriculture was state-subsidized, which has led to the use of virtually all arable land and left a highly fragmented renosterveld vegetation (Newton 2008). In post-apartheid time, fiscal governmental support has stopped and dryland farming became unproductive with many fields left abandoned. Exceptionally high level of habitat loss and

fragmentation has reduced chances to restore renosterveld to its original extent (Krug and Krug 2007). Today, abandoned fields show little recovery towards a historic vegetation state (Cramer *et al.* 2008). This is because ecosystem processes have seriously altered in renosterveld (Rebelo 1996). In general, conservation and restoration aims for renosterveld are difficult to pin point because it is uncertain how renosterveld looked like before European settlement (Rebelo 1995, Newton and Knight 2004) and how to manage it to this unknown state (Britton and Jackelman 1995). Also, because of anthropogenic influence and changed ecosystem drivers, the system is too degraded to recover via succession. The future of renosterveld species under global change is uncertain (Williams *et al.* 2005) and overuse of renosterveld is current practice (Duckitt 1995). Nutrient enrichment took place (Milton 2004), which favoured alien grass competition (Midoko-Iponga 2004, Midoko-Iponga *et al.* 2005) and diminished recolonization of degraded sites (Rebelo 1996, Shiponeni 2003, Shiponeni and Milton 2006) even if resource-intensive approaches had been implemented. Additionally, each stakeholder has different conservation and restoration aims (Hilderbrand *et al.* 2005). This array coupled with failed restoration experiments lead to the question if recent strategies are promising for the future. It seems necessary that remnants need novel conservation approaches and perspectives (Low and Jones 1995). A shift in restoration strategy and priority is required. Already, involved bodies look for a sustainable use of pristine and degraded renosterveld, such as flower production, game farming, ecotourism and biodiversity marketing (Krug and Krug 2007). However, because most renosterveld is situated on private land, only awareness of landowners, accompanied with establishment of conservancies and nature reserves as well as applying sensitive management practices, will have a significant impact on renosterveld restoration (C.A.P.E. 2000). Furthermore, it seems that conservation interests (Winter 2003, Winter *et al.* 2005) paired with financial incentives (Botha 2001) and stewardship programs (Kotze 2009) are increasing. Another interesting idea is the re-introduction of large game back into renosterveld and the usage of black rhino as flagship species (Rebelo 1995). However, high investments in new fencing would prohibit such efforts (C. Krug, pers. comm.). Other approaches to highlight the importance of renosterveld and to ensure its survival is linking with agriculture activities (Donaldson 2002), installation of renosterveld windbreaks (O'Farrell and Collard 2003, Botha *et al.* 2008) or implementation of carbon trade (renosterveld yield 80 t C ha<sup>-1</sup>, abandoned fields only the half, see Mills *et al.* 2003). Finally, conservation intervention must bridge the gap between knowing and doing (Knight and Cowling 2007) and implementation of the biodiversity network for the Cape Town area is urgently needed (Jackson 2004). Krug and Krug (2007) pointed out that

degraded renosterveld habitats may provide habitat for endangered species and could act as a buffer zone, corridor and stepping stones. To meet UN conservation goal of 26 % of former renosterveld area, additional land has to be claimed back, e.g. vineyards, wheat fields, pine plantations and degraded sites (Rebello 1995). Restored sites may also serve as diaspora source for further restoration attempts (Musil *et al.* 2005). Learning from restoration projects of other vegetation types and adapting those to local conditions could be an option, for example, applying a sowing approach (fynbos vegetation; Holmes 2005), installing of bird perches to enhance seed rain (tropical rainforest; Holl 1998) or pine clearing (European calcareous grasslands; Poschlod and Jordan 1992, Kiefer and Poschlod 1996). Research efforts are rewarding since the remaining renosterveld fragments have a rare and diverse flora and fauna. Remnants provide aesthetic value and recreation allowing for education and ecotourism, as well as being resources of medicinal and horticultural plants for local communities (Wood and Low 1993a). Furthermore, they act as nucleus for restoring adjacent degraded habitats. This outlook encourages scientists and stakeholders to ensure the survival, management and sustainable use of this unique vegetation type. This literature review highlights the relevance and requirements to deal with this matter, especially with regards to fragmentation and degradation of renosterveld remnants, as well as restoration potential of adjacent abandoned fields.

## **Methodology background**

In this thesis several methods were applied to describe the sampling area with its natural vegetation, soil parameter and soil seed bank in comparison to their degraded counterparts in abandoned fields and pine plantation. Furthermore, restoration experiments were established to enhance re-establishment of indigenous shrubs into degraded renosterveld. In order to evaluate fragmentation effects on genetic variation of species, plant material from several renosterveld fragments was collected and analyzed applying molecular methods. The following paragraphs show which particular methods chosen and why they were used.

### ***Vegetation surveys***

Several methods to describe vegetation exist for ecologists (Mueller-Dombois and Ellenberg 1974). Depending on the landscape context and region two different types have developed. American ecologists are faced with large tracks of homogenous vegetation and a systematic or randomized sampling methodology taking this into account (Küchler 1967). In Europe, a cultural landscape is evident (Ellenberg 1986) and subjective methods have developed

according to this rather small-scale heterogeneity (e.g. Braun-Blanquet approach). Lowland renosterveld of the Cape region of South Africa is scattered in small and isolated fragments, and high species richness and turnover within this vegetation is present (Rebelo *et al.* 2006). Therefore a subjective small-scale survey of the vegetation within these patches is necessary. Within plots, each living plant was identified with the help of field guides and by comparing herbarium specimens.

### ***Soil survey***

In order to evaluate chemical and physical soil parameters of a site, soil samples need to be collected. The heterogeneity of soils makes it necessary to use several probes that are pooled later, to characterize a certain plot (Schlichting and Blume 1966). This pooled probe will be dried and sieved resulting in a homogenous, stone and deposit free sample. This soil sample can now be analyzed for soil parameters and nutrients, such as pH, phosphorus, potassium, sum of exchangeable cations, nitrogen, carbon, conductivity, water holding capacity, and content of clay, silt and sand.

### ***Soil seed bank sampling and germination experiments***

For soil seed bank analysis several soil cores need to be derived from plots to be characterized. Usually 10 - 20 randomly taken replicate cores per plot are collected using machinery like a soil corer (Bakker *et al.* 1996). The dimension of the chamber needs to be known (diameter, depth, surface-area). The pooled soil samples are then put in paper bags, exposed to air for drying and stored dry at room temperature until further use. Depending on the sampling region (e.g. Central Europe) a stratification period is indicated, but was not necessary for samples from the Cape region. Later, soil samples are sieved with water to increase the seed/soil-ratio and to break the seed testa. After sieving, the samples were put on a sterile cultivation substrate in trays and watered to saturation. The cultivation trays are constantly watered with a water basin from underneath. Further irrigation can be manually carried out by a garden sprayer. Light and temperature regime also needs to be adjusted to the sampling region. The seedling emergence method was chosen to obtain species composition and abundances mirroring the in-field situation (Ter Heerdt *et al.* 1996). Emergence of seedlings was recorded. Seedlings were removed weekly and identified where possible with the help of field guides and herbarium material. Unknown seedlings were grown until identification was possible. In case of identification failure, the specimen was treated as a separate species, and genus, family or life form was noted. The soil or seed samples can also

be used for smoke-primer experiments, in order to test the effect of fire on germination (Brown 1993). Smoke-water is produced by use of impregnating absorbent paper with smoke-saturated water, which is dried later (e.g. smoke-primer at <http://finebushpeople.co.za>).

### ***Restoration experiments***

Abandoned agricultural fields and pine plantations are increasing features of altered ecosystems (Richardson et al. 1994, Young 2000, Cramer et al. 2007). Such potential restoration sites could increase remnant size and enhance connectivity between isolated natural habitats. However restoration success is constrained by abiotic and biotic factors (Saunders et al. 1991), as well as temporal and spatial dispersal of diaspores (Poschlod et al. 1998). One restoration approach aims to activate the still viable soil seed bank of pre-cultivation vegetation (Bakker et al. 1996). Several methods and their combination are available to achieve this aim, such as tilling, mowing, weeding, fire, cutting, sod cutting, and herbicide application. Problems can often be dominant alien species which are eradicated using the same methods. Another restoration approach uses external diaspores to facilitate re-establishment of indigenous species (Willems and Bik 1998). This can be realized via transplanting, sowing, animal dispersal (dung), installation of artificial bird perches and hay spreading.

The first experiment aimed to establish renosterveld pioneer shrub species *Otholobium hirtum* in an abandoned agricultural field via sowing strategy. This species is able to outcompete dominant grasses and its seeds are easily available from adjacent *O. hirtum* patches. Optimal germination requirements were evaluated in experiments. In-field experiments tested the several effects (e.g. scarification, competition, herbivory) of seedling germination and establishment.

In the second experiment, perches were erected in order to attract frugivorous birds, thereby enhancing seed deposition into abandoned agricultural fields. This bird-dispersed seeds are associated with vegetation sub-type of renosterveld, named “heuweltjies”. It aimed to re-establish this species spectrum in abandoned fields. Seed traps situated below each perch can be used to measure the input of bird faeces and bird-dispersed seeds (*sensu* Bullock et al. 2006). Prior to upcoming fruiting season traps can be removed and seedling establishment can be studied in vegetation and vegetation-free sites underneath each perch.

Former pine clear-cut experiments have been followed by immediate species recovery from soil seed banks (Kiefer and Poschlod 1996, Pärtel et al. 1998, Barbaro et al. 2001, Baba 2004, Bisteau et al. 2005, Blanckenhagen and Poschlod 2005, Dzwonko and Loster 2007).

Therefore chances are high that the performed pine clearing experiment could be successful. The region has several large stands of pine plantation that are problematic because of their invasive behavior. Therefore nature conservation authorities carried out a clear-cut of a pine plantation. This was observed with vegetation surveys and soil analyses at the cleared site and compared with adjacent pine plantation and pristine renosterveld vegetation.

### ***Population genetics***

In the early days of population genetics, isoenzymes were used to analyze genetic variation in species. Today more sophisticated methods are available such as the amplified fragment length polymorphism (AFLP). Sampling design (i.e. sampled populations within landscape) is a very important criterion within genetic studies and needs to be planned precisely. Renosterveld vegetation is highly fragmented and species could face loss of genetic variation due to reduction of population sizes and gene flow. Interestingly, three fragmentation degrees exist in the region and offer a unique study design.

For each population leaf material of individuals was collected and cooled on ice. Later they were placed into filter bags and dehydrated in silica gel. DNA was isolated from dried plant material of individual plants using CTAB method (cetyltrimethylammonium bromide, Rogers and Bendich 1994). Both, DNA isolation and AFLP method (Vos et al. 1995) were adapted as previously described (Reisch et al. 2005, Reisch 2008). After DNA precipitation, DNA pellets were vacuum-dried and dissolved in a mixture of Sample Loading Solution and CEQ Size Standard 400 (both Beckman Coulter). The fluorescence-labeled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter). Raw data were collected and analyzed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter). Data was exported showing synthetic gels with AFLP fragments for each primer combination separately from all studied individuals and analyzed in BIONUMERICS (Applied Maths). Files were examined for strong, clearly defined bands. Each band was scored across all individuals as either present or absent.

Finally, basic data structure consisted of a binomial (0/1) matrix, representing the scored AFLP markers. Genetic variation was calculated via POPGENE (Yeh et al. 1999) and tested for differences. The binomial matrix was subject to an analysis of molecular variance (AMOVA, Excoffier et al. 1992) using GENALEX (Peakall and Smouse 2006). Bayesian analysis was applied by using STRUCTURE (Pritchard et al. 2000) to determine number of group assignment (Evanno et al. 2005). Genetic relatedness between individuals, assorting

populations and differences between the three regions were analysed by principle coordinates analysis (PcoA). Calculations and plotting was performed in MVSP (Kovach 1999). A MANTEL test was conducted to test whether the matrix of pair-wise genetic distances taken from the AMOVA between populations was correlated with the matrix of geographical distances between populations (Mantel 1967, Sokal and Rohlf 1995).

## **2 Take it or leave it - degradation and restoration priorities of endangered West Coast Centre renosterveld**

### **Abstract**

Habitat transformation is increasing world-wide and has also left West Coast Centre Renosterveld in Cape Floristic Region of South Africa as a highly fragmented and endangered vegetation type. There are two main restoration options available in the area: abandoned fields and pine plantations. In both, remaining soil seed banks could be an important diaspore source for recovery of these degraded habitats. The Tygerberg Nature Reserve, north of Cape Town is the largest remaining renosterveld fragment (33°52'S, 18°35'E). Here, the extent of degradation on vegetation, soil parameters and soil seed banks of two abandoned fields and one pine plantation were examined and related to adjacent pristine renosterveld sites. With this, an evaluation of the restoration potential and priority of degraded habitats was possible. Results indicate that abandoned fields of renosterveld have a very low restoration potential due to depletion of indigenous soil seed bank, nutrient enrichment, and high cover and competition of alien grasses. However, restoration attempts of pine plantations showed high recovery potential and should be given priority in restoration. Here, a viable indigenous seed bank is present, and soil alteration and alien species are of less concern.

## Introduction

Land abandonment and pine stands are increasing features of anthropogenic altered ecosystems, and potential sites for restoration and conservation (Richardson *et al.* 1994, Young 2000, Cramer *et al.* 2007). Restoration of abandoned agricultural fields and pine stands would increase remnant size, create buffer zones and enhance their connectivity. However, this is challenging because restoration success depends on abiotic and biotic factors (Saunders *et al.* 1991), as well as temporal and spatial dispersal of diaspores (Poschlod *et al.* 1998). Furthermore, former cultivation regimes can direct plant succession after abandonment (Gibson and Brown 1991), due to elevated soil nutrient status caused by fertilizer application (Gough and Marrs 1990) and presence of ruderal species (Grime 1979). One way to overcome these problems is to activate the “memory” of pre-cultivation vegetation, which could still be viable in seed banks (Bakker *et al.* 1996), as well as to use external diaspore sources, which could facilitate recovery of indigenous species (Willems and Bik 1998). European studies on abandoned fields and afforestations show limited short-term success of re-establishment of indigenous plant species (Blanckenhagen and Poschlod 2005, Römermann *et al.* 2005, Buisson *et al.* 2006). Nevertheless, successful long-term succession is possible under certain circumstances (Karlík and Poschlod 2009, Piqueray *et al.* 2010). Clear-cut experiments of pine stands have been followed by immediate species recovery from soil seed banks (Kiefer and Poschlod 1996, Pärtel *et al.* 1998, Barbaro *et al.* 2001, Baba 2004, Bisteau *et al.* 2005, Blanckenhagen and Poschlod 2005, Dzwonko and Loster 2007).

The Cape Floristic Region at the south-western tip of Africa is the most species-rich region in the outer-tropics, the smallest of all six Floral Kingdoms (Takhtajan 1969) and a biodiversity hotspot (Myers *et al.* 2000, Myers 2001). Until recently, research and restoration attempts have focused on fynbos, the main vegetation type in the region occurring on nutrient-poor soils. In contrast, lowland renosterveld vegetation is found on nutrient-rich soils making it suitable to dry land agriculture. Large scale habitat transformation took place in the Cape Lowlands with the beginning of European settlement from 1652. This transformation, coupled with alien vegetation and urbanization, left only 10 % of former West Coast Centre renosterveld that is now the most endangered vegetation type of South Africa and occurs only in small fragments (Cowling and Heijnis 2001, von Hase *et al.* 2003b, Rebelo *et al.* 2006). Unfortunately, conservation status is very poor with only 0.6 percent of renosterveld under protection (Cowling *et al.* 1999a). This is despite of its global biodiversity importance (Cowling and Pierce 1999) and regional conservation priority (Rebelo 1997). Abandonment of agricultural fields began in the 1980s and currently renosterveld remnants are often

surrounded by such fields, which are characterized by non-indigenous species, mostly southern European arable weeds and pasture grasses. Estimations suggest that one percent of former renosterveld are nowadays made of abandoned fields and pine plantations (von Hase *et al.* 2003b).

These areas are potential restoration sites and have therefore moved onto the research agenda in order to increase renosterveld areas (Krug and Krug 2007). However, information on soil seed banks in renosterveld are underrepresented despite their importance in providing information about the local species pool and the restoration potential of degraded renosterveld sites. In general, seed dispersal is limited in fragmented landscapes (Poschlod *et al.* 1996, Bakker and Berendse 1999). Nevertheless, it appears that except for geophytes seed dispersal into abandoned fields is not limited, instead competition and grazing are limiting factors for seedling survival and establishment (Shiponeni 2003, Midoko-Iponga *et al.* 2005). Furthermore, fast-growing alien annual grasses have a higher capacity of invading nutrient-enriched habitats compared to indigenous plants (Sharma *et al.* 2010), which can lead to increased dominance.

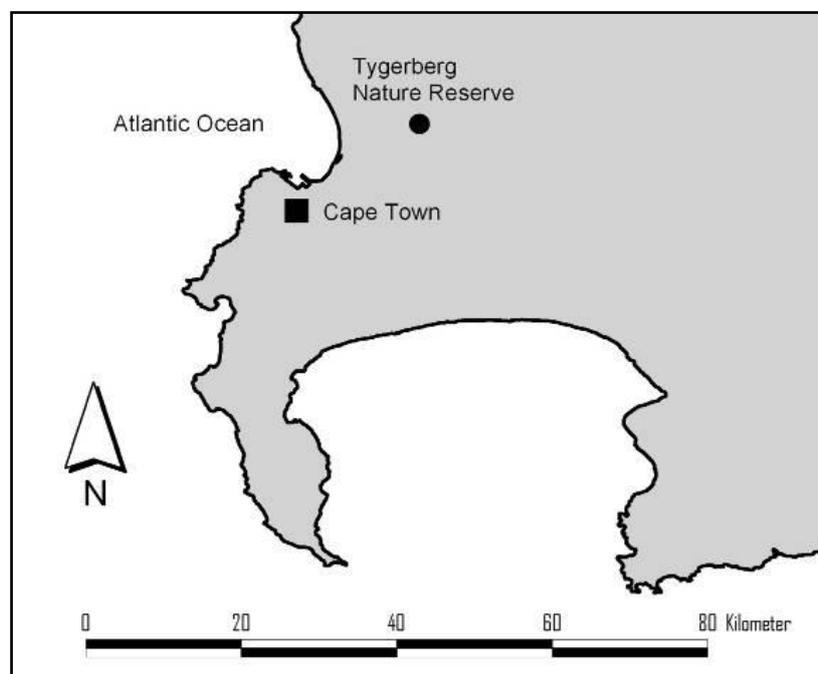
Initial soil seed bank studies in the area have concentrated on edge effects of renosterveld fragments within an agricultural landscape and the seed influx of alien grasses (Shiponeni 2003, Muhl 2008). A small-scale seed bank study in mountain renosterveld showed little restoration potential of abandoned fields (Saayman and Botha 2008). Both studies have mentioned the problem of slow succession of abandoned fields towards a proposed renosterveld status. A further reason for degradation of renosterveld is pine plantation. Although suggestions for pine clearing (De Villiers *et al.* 2005) and observations exist (Boucher, pers. comm.), there are no data on soil seed banks in pine plantations in the Cape region available.

The first objective was to describe abiotic (soil chemistry) and biotic (vegetation, soil seed bank) conditions of three renosterveld patches and adjacent degraded habitats (i.e. two abandoned fields and one pine plantation). Specifically, it was predicted to measure a significant impact of former agriculture and forestry in degraded habitats, such as lower species numbers and more alien species, altered soil parameters, and depleted soil seed bank. The gathered information should deduce the potential of degraded habitats for re-development into a renosterveld surrogate, in order to set restoration priorities.

## Material and Methods

### *Study area and sites*

The study was performed at Tygerberg Hills (33°52`S, 18.35`E) in the Cape Lowlands of South Africa (Fig. 4). The area has a typical Mediterranean-type climate with a winter-rainfall and summer-drought regime. Records of European influence date back to 1655 when Jan Wintervogel first explored the Tygerberg area. Freehold farms with corn-fields, vineyards, wheat and gardens, sheep and cattle farming were established in the 1700's. The area was partly farmed and ploughed until 1938 and wheat, barley, oats and rye, and vineyards were the main farming products. Urban settlement took place from 1945 (J. Kuyler, pers. comm.). Nowadays, Tygerberg hills and the Tygerberg Nature Reserve are important conservation sites (Jarman 1986), as well as an eco-tourism and education centre (Wood and Low 1993a). Two renosterveld categories, namely pristine and degraded renosterveld, each comprising 30 plots (2 x 2 m) were surveyed. Specifically, three pristine renosterveld sites were examined (RV1, RV2, RV3, 3 x 10 plots). Adjacent to these sites degraded renosterveld were studied, namely abandoned fields (AF1, AF2, 2 x 10 plots) and a pine plantation (PP, 10 plots). Both, abandoned fields and pine plantation have been used as such for many decades (J. Kuyler, pers. comm.).



**Fig. 4.** Map of the south-western Cape of South Africa. Study site of the Tygerberg Nature Reserve (dark dot).

*Vegetation and soil survey*

Within plots, each living plant was identified with the help of various field guides (Kidd 1983, Manning and Goldblatt 1996, Goldblatt and Manning 2000, Trinder-Smith and Levyns 2003) and by comparing herbarium specimens of the Tygerberg Nature Reserve Herbarium. Species identification was carried out in flowering seasons (July-October 2007 and 2008). In case of identification failure, the specimen was treated as a separate species, and genus, family or life form was noted. Five soil samples (app. 200 g each) per plot were taken and pooled in August 2007. After drying and sieving (200  $\mu\text{m}$ ), each soil sample was analysed for soil nutrients (pH, phosphorus, potassium, sum of exchangeable cations, nitrogen and carbon, silt, sand, and stone) at BemLab (Pty) LTD Somerset West, South Africa. Furthermore, one soil sample per plot was taken to analyse water holding capacity (Steubing and Fangmeier 1992).

*Soil seed bank sampling and germination*

Soil seed bank sampling took place in March and April 2007. In total, 1200 soil cores were taken from plots where vegetation surveys were made. For each land use type 20 replicate cores (4 cm diameter, 10 cm depth, surface-area 12.5 cm<sup>2</sup>, volume 125 cm<sup>3</sup>) were taken at random (Bakker *et al.* 1996) and divided in two sub-samples of different depth (0-5 cm, 5-10 cm). The samples were put in paper bags, exposed to air for a week for drying and stored dry at room temperature until further use. Later, soil samples were sieved with water (mesh size 5 mm) to increase the seed/soil-ratio and to break the seed testa. The fraction larger than 5 mm was visually checked for remaining seeds and bulbs. After sieving, the samples were divided by half and put on a sterile cultivation substrate (sand plus white peat with clay, soil ratio 1.5:6, pH (CaCl<sub>2</sub>): 5.8-6, salt (KCl): 1.5g/l, N (CaCl<sub>2</sub>): 150-180mg/l, P<sub>2</sub>O<sub>5</sub>: 150-200mg/l, K<sub>2</sub>O: 210-250 mg/l) in plastic trays (60 cm x 40 cm) and watered to saturation. One sample was used for the germination experiment, the remaining sample for the smoke-primer experiment (chapter three, page 36). The cultivation trays were constantly watered through a plaited glass fibre thread by a water basin from underneath for a month. Further irrigation was manually carried out by a garden sprayer. Light and temperature regime was 14 h light / 22 °C and 10 h dark / 10 °C (Brown and Botha 2004). Germination trials were conducted in the greenhouses of the Botanical Institute at the University of Regensburg and the seedling emergence method was chosen to obtain species composition and abundances (Ter Heerd *et al.* 1996). Emergence of seedlings was recorded; seedlings were removed weekly and identified where possible with help of field guides and herbarium material (Kidd 1983, Manning and Goldblatt 1996, Goldblatt and Manning 2000, Trinder-Smith and Levyns 2003).

Unknown seedlings were grown until identification was possible. In case of identification failure, the specimen was treated as a separate species, and genus, family or life form was noted.

### *Data analyses*

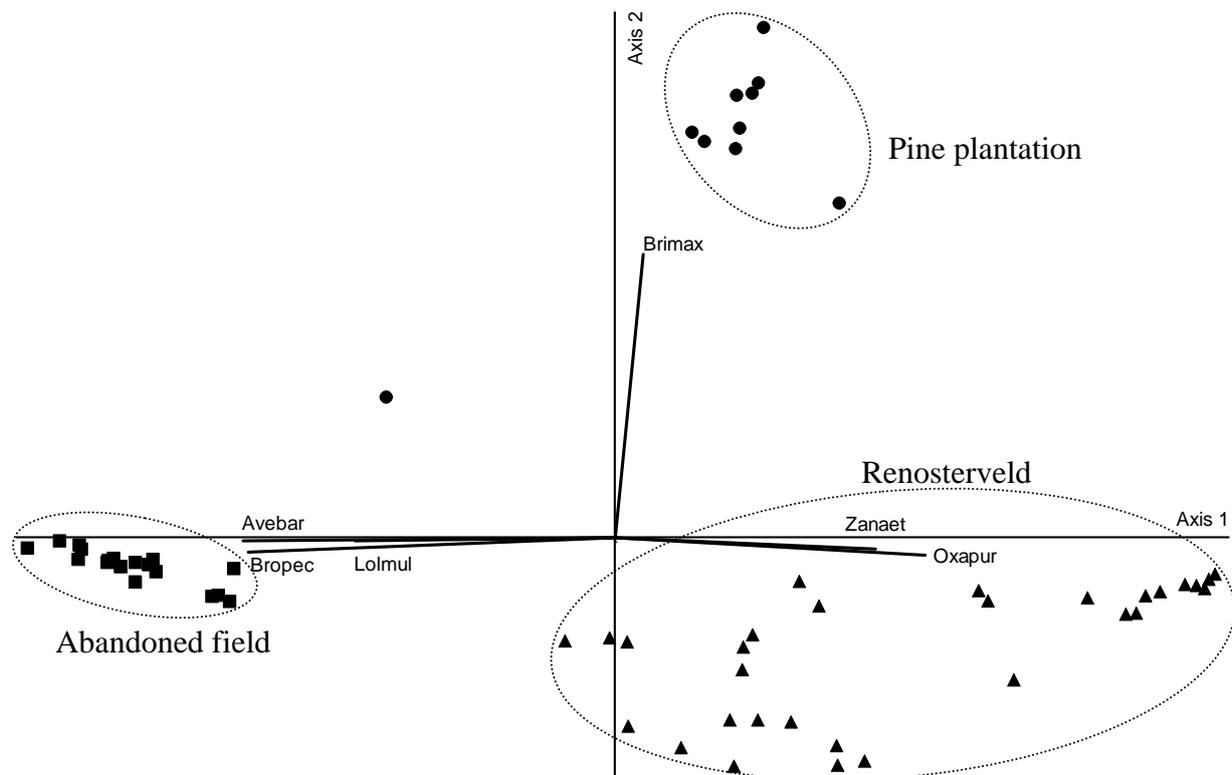
Once the collected soil, vegetation and seed bank data had normal distribution and homogenous variances, statistical comparison was carried out via single-factor ANOVA, post-hoc LSD-test or pair-wise *t* tests. Remaining parametric data were analysed using Mann-Whitney *U* and Wilcoxon tests. All tests were computed with SPSS for Windows (SPSS Inc., version 15.0). Furthermore, vegetation and soil data were analysed via a detrended component analysis (DCA) using PC-Ord 4.0 (McCune and Mefford 1999). Species richness (total and mean number of species, evenness) was calculated using the same program. Vegetation data combined with abiotic factors were analysed using a DCA in order to identify abiotic parameters that have influenced species composition. Vegetation data constituted the main matrix and soil chemistry was standardised to become second matrix. Parameters of the soil seed bank (i.e. seed abundance by species) were analysed and calculated. Vegetation data combined with soil seed bank data were then analysed using a DCA with power-transformed data and contained a combined matrix of both data sets.

## **Results**

### *Vegetation survey*

A detrended component analysis showed that examined renosterveld communities at Tygerberg Hills are characterized by indigenous geophytes *Oxalis purpurea* and *Zantedeschia aethiopica* (Fig. 5). Abandoned fields are characterized by alien grass species, such as *Avena barbata*, *Bromus pectinatus*, and *Lolium multiflorum*. Pine plantation shows a strong relation to alien grass *Briza maxima*. Total species number and mean species number per plot differed widely between sites (Table 1). With 15-21 species, abandoned fields had lower species number than found in pine plantation (59 spp.) and pristine renosterveld (36-93 spp.). Significant higher mean species number per plot are found in renosterveld 2 and 3 (25.9 spp., 27.8 spp.) compared to renosterveld 1 (15.3 spp.) and pine plantation (13.6 spp.). Lowest mean species number per plot is found in abandoned fields (7.8 -8.3 spp.). Species evenness is similarly high between renosterveld sites (~0.75) and significantly different from abandoned fields (~0.71). Pine plantation showed the lowest evenness value (0.52). In total 169 species were found in above ground vegetation (Appendix 1). Two red data species appeared during

the vegetation survey, namely *Hermannia rugosa* and *Asphalatus acanthoides*, in renosterveld 2 and 3, respectively.



**Fig. 5. DCA ordination of above-ground vegetation at Tygerberg Nature Reserve.**

Renosterveld (triangles), abandoned field (squares), pine plantation (circles), matrix: 169 species, 10 quadrates of 6 sites, increment (axis 1: 0.42, axis 2: 0.15),  $r^2$ -cut-off-value=0.4, species correlation axis 1: *Avena barbata* (Avebar,  $r^2=0.63$ ), *Bromus pectinatus* (Bropec,  $r^2=0.62$ ), *Lolium multiflorum* (Lolmul,  $r^2=0.44$ ), *Oxalis purpurea* (Oxapur,  $r^2=0.52$ ), *Zantedeschia aethiopica* (Zanaet,  $r^2=0.44$ ), species correlation axis 2: *Briza maxima* (Brimax,  $r^2=0.48$ ). log: 4.73.

**Table 1. Species and seed number in vegetation and soil seed bank.**

Renosterveld (RV), abandoned field (AF), pine plantation (PP). Means and standard error of means are given. Significant differences of ANOVA-analysis with LSD post-hoc test between sites ( $n=10$ ) are indicated with different letters. Evenness and Sørensen index is given as diversity and similarity measure. Soil horizon comparison was done via joined  $t$ -test. Significance level  $p<0.01$  (\*),  $p<0.001$  (\*\*).

|   | RV1                      | RV2                     | RV3                    | AF1                     | AF2                      | PP                      |
|---|--------------------------|-------------------------|------------------------|-------------------------|--------------------------|-------------------------|
| <b>Vegetation</b>                                   |                          |                         |                        |                         |                          |                         |
| Total no. of species                                | 36                       | 78                      | 93                     | 15                      | 21                       | 59                      |
| Mean no. of species per plot **                     | 15.3±0.2 <sup>b</sup>    | 25.9±0.6 <sup>c</sup>   | 27.6±0.6 <sup>c</sup>  | 7.8±0.2 <sup>a</sup>    | 8.3±0.3 <sup>a</sup>     | 13.6±0.4 <sup>b</sup>   |
| Evenness **   | 0.80±0.03 <sup>c</sup>   | 0.79±0.02 <sup>c</sup>  | 0.75±0.03 <sup>c</sup> | 0.71±0.01 <sup>b</sup>  | 0.72±0.03 <sup>b</sup>   | 0.52±0.05 <sup>a</sup>  |
| <b>Soil seed bank</b>                               |                          |                         |                        |                         |                          |                         |
| Total no. of species                                | 37                       | 50                      | 40                     | 32                      | 37                       | 39                      |
| Mean no. of species per sample (0-10cm)**           | 19.7±0.76 <sup>a</sup>   | 18.0±1.67 <sup>ac</sup> | 19.0±1.00 <sup>a</sup> | 14.8±1.23 <sup>bc</sup> | 15.2±1.36 <sup>bc</sup>  | 13.9±1.13 <sup>b</sup>  |
| Evenness *  | 0.70±0.02 <sup>a</sup>   | 0.84±0.03 <sup>b</sup>  | 0.80±0.03 <sup>b</sup> | 0.72±0.05 <sup>a</sup>  | 0.63±0.06 <sup>c</sup>   | 0.77±0.04 <sup>b</sup>  |
| Total no. of seeds                                  | 1962                     | 621                     | 872                    | 904                     | 1447                     | 599                     |
| Mean no. of seeds per sample (63cm <sup>3</sup> )** | 196.2±18.46 <sup>c</sup> | 62.1±17.55 <sup>a</sup> | 87.2±9.99 <sup>a</sup> | 90.4±11.96 <sup>a</sup> | 114.7±21.67 <sup>b</sup> | 59.9±10.31 <sup>a</sup> |
| Total no. of seeds per 1m <sup>2</sup> **           | 15634±1471 <sup>c</sup>  | 4948±1398 <sup>a</sup>  | 6948±795 <sup>a</sup>  | 7203±953 <sup>a</sup>   | 11530±1727 <sup>b</sup>  | 4773±821 <sup>a</sup>   |
| Mean no. of seeds per 1m <sup>2</sup> (0-5cm)**     | 8383±871 <sup>bc</sup>   | 3578±1092 <sup>a</sup>  | 5219±796 <sup>a</sup>  | 6191±934 <sup>ab</sup>  | 10016±1512 <sup>c</sup>  | 4199±791 <sup>a</sup>   |
| Mean no. of seeds per 1m <sup>2</sup> (5-10cm)**    | 7251±770 <sup>c</sup>    | 1371±361 <sup>ab</sup>  | 1729±336 <sup>ab</sup> | 1012±232 <sup>ab</sup>  | 1514±351 <sup>ab</sup>   | 574±80 <sup>a</sup>     |
| Mean no. of seeds (0-5cm vs. 5-10cm)**              | n.s                      | *                       | *                      | **                      | **                       | *                       |
| Sørensen index of vegetation and seed bank          | 0.27                     | 0.32                    | 0.27                   | 0.43                    | 0.45                     | 0.18                    |

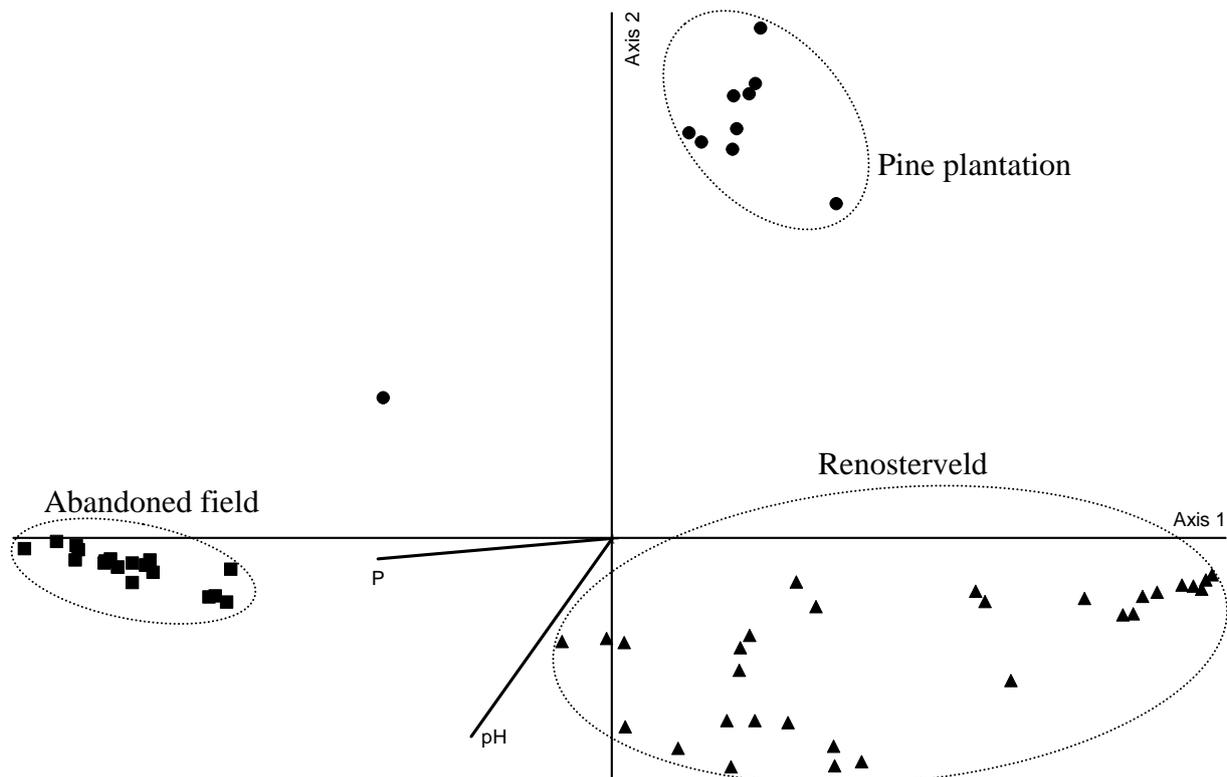
*Soil survey*

The results of soil analysis between renosterveld 1, 2 and abandoned field 1, 2 (comparison 1) showed statistically significant differences at all parameters except for potassium, carbon, C:N, conductivity and water holding capacity (Table 2). A similar picture became evident between renosterveld 3 and pine plantation site (comparison 2), with all parameters showing statistically significant changes, except for potassium, carbon and sand fraction. A detrended component analysis of vegetation and soil data revealed that phosphorus is positively correlated with abandoned fields (Fig. 6). The pH-level is negatively correlated with pine plantation.

**Table 2. Comparison of chemical and physical soil parameters in different land use types.**

Mean, standard error of mean and test value of *t*-test (*T*) or Mann-Whitney *U* test (*U*) with *p*-value (\**p* < 0.05, \*\**p* < 0.01, \*\*\* *p* < 0.001, n.s. – not significant) are given. Cation exchange capacity (CEC) with T-value: sum of exchangeable sodium, potassium, calcium, magnesium and hydrogen. Water holding capacity (WHC). Conductivity (Cond.). Sampling sizes: comparison 1 (n=20), comparison 2 (n=1). Sampling sizes for clay, silt and sand: comparison 1 (n=10), comparison 2 (n=5).

| Parameter     | Comparison 1         |                          |                      | Comparison 2   |                    |                     |
|---------------|----------------------|--------------------------|----------------------|----------------|--------------------|---------------------|
|               | Renosterveld<br>1, 2 | Abandoned<br>fields 1, 2 | Statistics           | Renosterveld 3 | Pine<br>plantation | Statistics          |
| pH            | 5.75±0.09            | 6.06±0.70                | <i>T</i> =-2.76 **   | 5.41±0.07      | 4.88±0.07          | <i>U</i> =4.5 ***   |
| P (mg/kg)     | 13.05±1.12           | 25.15±2.36               | <i>U</i> =48.0 ***   | 5.5±0.45       | 8.4±0.40           | <i>T</i> =-4.8 ***  |
| K (mg/kg)     | 308.35±19.9          | 301.0±15.7               | <i>T</i> =0.29 n.s.  | 259.4±14.3     | 294.8±10.5         | <i>T</i> =-2.0 n.s. |
| N (%)         | 0.29±0.02            | 0.19±0.01                | <i>T</i> =4.26 ***   | 0.19±0.01      | 0.35±0.03          | <i>U</i> =0.0 ***   |
| C (%)         | 1.97±0.06            | 1.73 ±0.11               | <i>T</i> =1.8 n.s.   | 2.17±0.05      | 2.23±0.31          | <i>U</i> =3.8 n.s.  |
| C:N           | 7.52±0.60            | 8.97±0.41                | <i>T</i> =-2.0 n.s.  | 11.4±0.42      | 6.22±0.41          | <i>T</i> =8.77 ***  |
| CEC (cmol/kg) | 13.32±0.97           | 9.98±0.49                | <i>T</i> =3.08 **    | 8.4±0.48       | 15.37±0.84         | <i>U</i> =0.0 ***   |
| Cond. (Ohm)   | 1250±69.0            | 1355±45.8                | <i>T</i> =-1.27 n.s. | 1774±55.8      | 1054±55.7          | <i>T</i> =9.1 ***   |
| WHC (%)       | 250.30±3.60          | 256.64±4.63              | <i>T</i> =1.03 n.s.  | 265.64±3.39    | 239.15±7.07        | <i>T</i> =-3.1 **   |
| Clay (%)      | 1.28±0.13            | 3.3±0.42                 | <i>U</i> =10.0 **    | 1.00±0.00      | 2.12±0.28          | <i>T</i> =4.0 *     |
| Silt (%)      | 6.86±1.19            | 16.2±1.03                | <i>U</i> =3.5 ***    | 11.4±1.40      | 4.8±1.20           | <i>T</i> =-3.58 **  |
| Sand (%)      | 92.0±1.17            | 80.7±1.31                | <i>U</i> =2.0 ***    | 87.6±1.40      | 92.8±1.20          | <i>T</i> =2.82 n.s. |



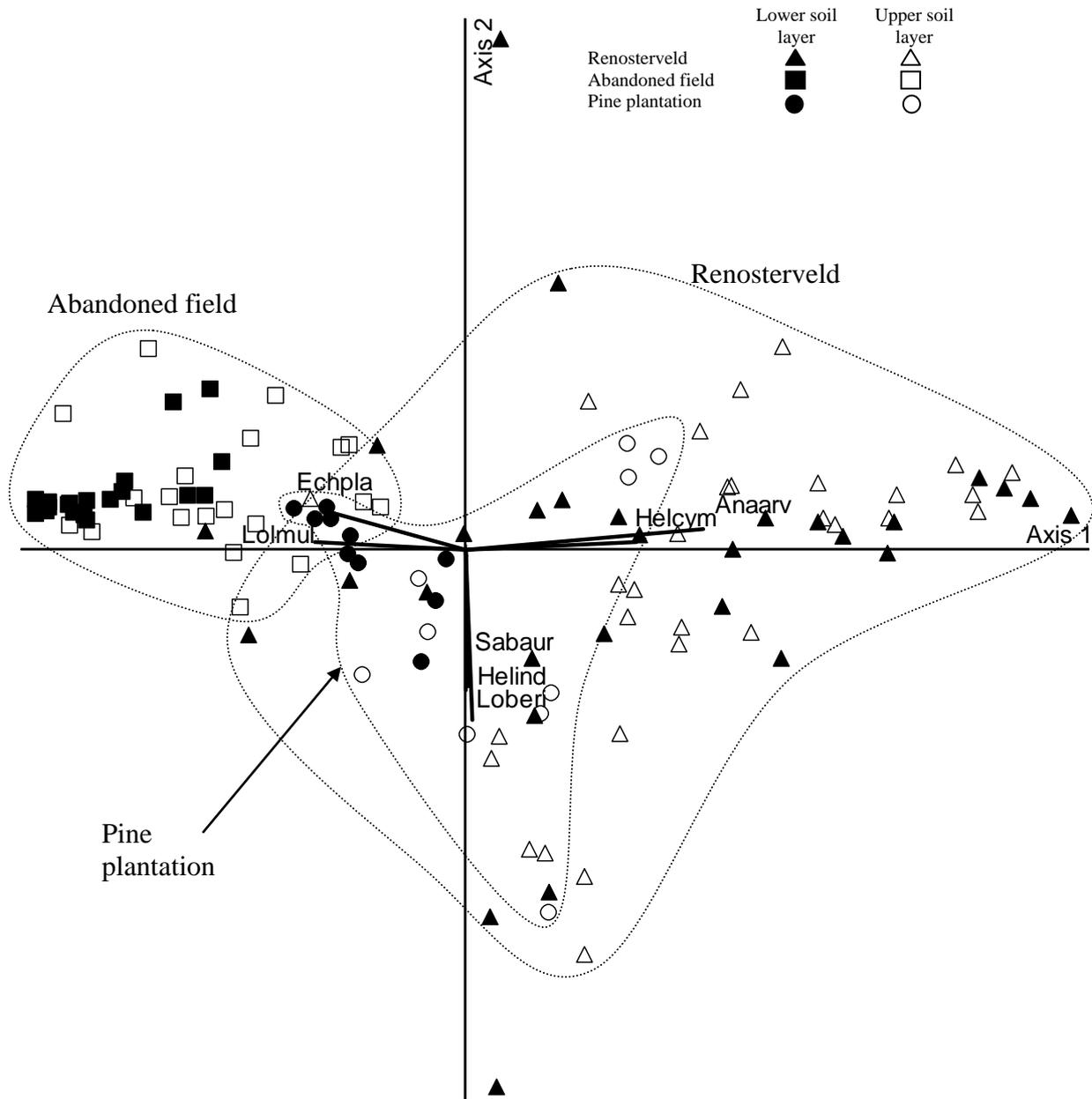
**Fig. 6. DCA ordination of vegetation and soil parameters at Tygerberg Nature Reserve.**

Matrix: 169 species, 10 quadrates of 6 sites. Renosterveld (triangles), abandoned field (squares), pine plantation (circles), increment (axis 1: 0.42, axis 2: 0.15),  $r^2$ -cut-off-value=0.3, parameters correlated with axis 1: P-level ( $r^2=0.4$ ), parameters correlated with axis 2: pH-level ( $r^2=0.34$ ), Log: 4.83. Physical soil parameters were excluded from the analysis. Soil parameters were standardized.

### *Soil seed bank survey*

During the germination experiment, 6405 seedlings of 91 taxa emerged from soil samples (Table 1). Eighty-one species were identified and assigned to 30 families, leaving 10 spp. unidentified. Main families represented in the soil seed bank were Asteraceae and Poaceae, with 21 spp. and 12 spp., respectively. A detailed overview of emerged seedlings is given (Appendix 1), as well as a list of species from vegetation only (Appendix 2). Site comparisons revealed significantly higher mean species number in pristine renosterveld (18.0 – 19.7 spp.) compared to degraded sites (13.9 - 15.2 spp.). No such pattern was found for species evenness, mean and total number of seeds throughout soil horizons. Significantly higher seed densities occurred in the upper soil layer (0-5 cm) compared to the lower soil layer (5-10 cm), except for RV1. A detrended component analysis showed that seed bank of renosterveld communities are mainly characterized by indigenous herb species, such as *Lobelia erinus*, *Helichrysum indicum* and *Sabea aurea*, *Helichrysum cymosum*. Abandoned fields are characterized by alien grass and herb species *Lolium multiflorum* and *Echium plantagineum* (Fig. 7). The pine plantation showed an intermediate position with greater similarities to pristine renosterveld. Species with a significantly heterogeneous depth distribution over the

sampled soil profile are shown in Table 3. Emerged seedlings from the soil seed bank were depicted by life form composition, which shows that proportions of herbs (23-30 spp.), shrubs (1-6 spp.), grasses (4-10 spp.) and geophytes (3-6 spp.) are very similar amongst sites. A high proportion of alien species is present within all sites (30-56 %).



**Fig. 7. DCA ordination of soil seed bank at Tygerberg Nature Reserve.**

Matrix: 91 species, 20 soil samples of 6 sites. Renosterveld (triangles), abandoned field (squares), pine plantation (circles), upper soil layer (empty figures), lower soil layer (full figures), increment (axis 1: 0.28, axis 2: 0.09),  $r^2$ -cut-off-value=0.25. Species correlated with axis 1: *Anagallis arvensis* ssp. *cerulea* (Anaarv,  $r^2=0.45$ ), *Helichrysum cymosum* (Helcym,  $r^2=0.32$ ), *Lolium multiflorum* (Lolmul,  $r^2=0.29$ ), *Echium plantagineum* (Echpla,  $r^2=0.27$ ). Species correlated with axis 2: *Lobelia erinus* (Loberi,  $r^2=0.33$ ), *Helichrysum indicum* (Helind,  $r^2=0.27$ ), *Sabea aurea* (Sabaur,  $r^2=0.26$ ). log: 3.89.

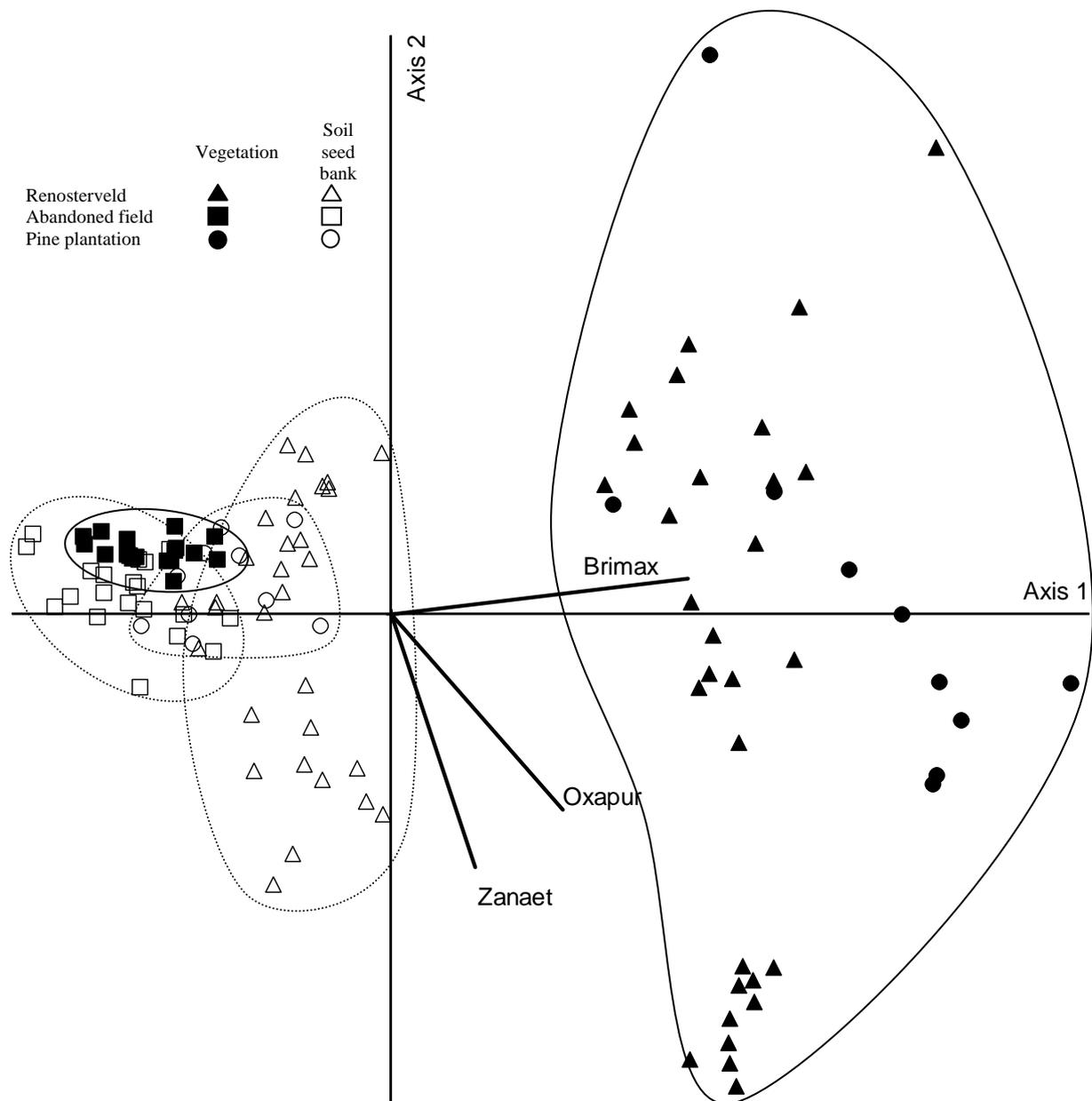
**Table 3. Soil seed bank data on depth distribution, life forms and alien species.**

List of species with a significantly heterogeneous depth distribution over the sampled soil profile. Taxon name followed by significant Z-values derived from Wilcoxon test (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ ). Renosterveld (RV), Abandoned field (AF), Pine plantation (PP).

| Category  | RV1    | RV2    | RV3    | AF1     | AF2     | PP     |
|---|--------|--------|--------|---------|---------|--------|
| <b>Species with higher seed density in upper layer (0-5 cm)</b>   |        |        |        |         |         |        |
| <i>Athenasia trifurcata</i>                                       | -2.1*  | -      | -      | -       | -       | -      |
| <i>Avena barbata</i>  | -      | -      | -2.0*  | -2.8**  | -2.7**  | 2.8**  |
| <i>Bromus pectinatus</i>  | -      | -      | -      | -       | -       | -2.0*  |
| <i>Echium plantagineum</i>  | -      | -      | -      | -       | -2.3*   | -      |
| <i>Helichrysum pandurifolium</i>                                  | -      | -      | -      | -2.4*   | -       | -2.7** |
| <i>Helichrysum teretifolium</i>                                   | -      | -      | -2.4*  | -2.0*   | -       | -      |
| <i>Lolium multiflorum</i>   | -      | -      | -      | -2.8**  | -2.8*   | -      |
| <i>Nidorella foetida</i>  | -      | -      | -      | -       | -       | -2.2*  |
| <i>Oxalis compressa</i>   | -      | -      | -      | -2.4*   | -2.0*   | -      |
| <i>Sonchus olearacea</i>  | -      | -      | -      | -       | -       | -2.2*  |
| <b>Species with higher seed density in lower layer (5-10 cm)</b>  |        |        |        |         |         |        |
| <i>Helichrysum cymosum</i>  | -2.5*  | -      | -      | -       | -       | -      |
| <b>Species number according to life forms (and alien species)</b> |        |        |        |         |         |        |
| Herb  | 23 (9) | 30 (7) | 19 (4) | 21 (11) | 23 (12) | 23 (6) |
| Shrub   | 6 (0)  | 6 (0)  | 8 (0)  | 3 (0)   | 3 (0)   | 6 (1)  |
| Grass   | 4 (3)  | 8 (5)  | 9 (5)  | 5 (4)   | 7 (5)   | 7 (6)  |
| Geophyte  | 4 (0)  | 6 (0)  | 4 (0)  | 3 (0)   | 4 (0)   | 3 (0)  |
| Alien species infestation   | 30 %   | 24 %   | 23 %   | 47 %    | 46 %    | 33 %   |

#### *Combination of soil seed bank and vegetation data*

A detrended component analysis on a power-transformed matrix of vegetation and soil seed bank data revealed a distinct composition between renosterveld and pine plantation vegetation, and old field vegetation and soil seed banks (Fig. 8). Above ground species composition differs from that of seed banks, except for old fields. Alien grass *Briza maxima* and indigenous geophytes *Zantedeschia aethiopica*, *Oxalis purpurea* are characteristic for pristine renosterveld and pine plantation. Sørensen index shows a low level of similarity (Table 1) between soil seed bank and above ground abandoned field vegetation (~0.44), especially in renosterveld (~0.29) and pine plantation (0.18).



**Fig. 8. DCA ordination of above ground vegetation cover and seed bank data.**

Renosterveld (triangles), abandoned field (squares), pine plantation (circles), soil seed bank (empty figures, dotted line), vegetation (full figures, full line). Vegetation cover in percentage and seed bank data in number of seeds in a power-transformed matrix: 215 taxa, 120 plots, log 4.46. Increment (axis 1: 0.38, axis 2: 0.08, r-cut-off-value=0.5). Species correlated with axis 1: *Briza maxima* (Brimmax,  $r^2=0.57$ ), species correlated with axis 2: *Oxalis purpurea* (Oxapur,  $r^2=0.31$ ), *Zantedeschia aethiopica* (Zanaet,  $r^2=0.48$ ).

## Discussion

The study results revealed underlying factors for slow recovery of abandoned fields at the Tygerberg Nature Reserve, such as nutrient enrichment, depleted indigenous soil seed bank and high abundances of alien competitive species. In contrast, pine plantation showed high restoration potential due to less degraded chemical soil parameters, viable indigenous soil seed bank, and less alien species infestation.

### *Vegetation analysis*

The vegetation analysis showed a clear separation and distinct species composition between pristine, abandoned fields and pine plantation. The species responsible for these differences are mainly alien grasses. Vegetation of pine plantation is more similar to pristine renosterveld sites, which are characterized by indigenous shrubs and geophytes. Furthermore, significantly more species were counted in pristine habitats compared to degraded sites and the prediction of a higher species number in the pristine sites was confirmed.

It is known that last crop influences succession of old fields (Myster and Pickett 1990) and alien grass species were used in the area (J. Kuyler, pers. comm.). Results indicate a low re-establishment of indigenous species in degraded habitats, which is a phenomenon also known for other vegetation types (Eliason and Allen 1997, Humphrey and Schupp 2004). Previous research on abandoned fields in renosterveld has shown that grass competition had a stronger effect on establishment than herbivory (Midoko-Iponga 2004, Midoko-Iponga *et al.* 2005). Other barriers for establishment of native shrub species included root competition between alien and indigenous species (Davies 1985) and high soil compaction of degraded habitats (Bassett *et al.* 2005). The negative effect of herbivory (i.e. seed predation, seedling removal, trampling) can diminish establishment of species (Davies 1985, Bonser and Reader 1995, Jones and Esler 2004). Vesicular-arbuscular mycorrhizal (VAM) infection is high in renosterveld compared to fynbos and strandveld (Allsopp and Stock 1994). Lack of VAM could be an additional reason for establishment failure of indigenous shrub in abandoned fields. Additionally, re-vegetation progress might depend on slope aspect and could be a reason for limited success (Wood and Low 1993b).

### *Soil analysis*

Comparison of pristine and degraded renosterveld revealed many significant differences in soil parameters that indicate a strong impact of former agricultural activities (e.g. increased phosphorus level by fertilizer-use) and forestry (e.g. acidification by pine litter). Soil

degradation after agriculture is also reported from abandoned fields in the region (Memiaghe 2008) and from the Mediterranean Basin (Römermann *et al.* 2005). In general, recovery of altered habitats is constrained by eutrophication problems (Bakker and Berendse 1999). Phosphorus and nitrogen enrichment is of special concern because it can enhance survival and invasion of alien species (Allcock 2002, Leishman and Thomson 2005, Stanway 2007). In this context, abundant alien grasses are more effective competitors (i.e. phosphorus uptake) compared to native shrubs (Caldwell *et al.* 1985, Caldwell *et al.* 1987, Suding *et al.* 2004). Elevated phosphorus levels on degraded land were found in renosterveld vegetation near Nieuwoudtville (O'Farrell and Collard 2003). Following nitrogen enrichment, the invasive annual grass *Avena fatua* was able to outcompete indigenous species in a pot experiment (Sharma *et al.* 2010). Although phosphorus level is significantly elevated in pine plantation (8.4 mg/kg), it does not reach the magnitude of abandoned fields (25.15 mg/kg).

#### *Soil seed bank analysis*

Soil seed bank analysis showed a distinct but not strong grouping between renosterveld and abandoned fields. Pine plantation is most similar to renosterveld habitats. Higher seed densities were found in the upper soil layer. An exception was renosterveld 1 with even distribution of seed numbers between upper and lower soil layer. This could be due to high bioturbation activity in this area (pers. observ.). Only eleven species had a significant heterogeneous depth distribution over the sampled soil profile and are responsible for this impression. Life form composition and high proportion of alien species were similar amongst sites. The distinct grouping and depleted indigenous soil seed bank of abandoned fields mean that they are unsuitable for restoration attempts that rely on diaspores in soil. In contrast, pine plantation had more similarities with pristine renosterveld and viable indigenous seed bank with great restoration options. Seed number of pristine renosterveld (4.948-15.633/m<sup>2</sup>) and abandoned fields (4.812-11.530/m<sup>2</sup>) was similarly high and also had a similar magnitude as previously found in renosterveld (pristine renosterveld 16.429/m<sup>2</sup>, abandoned fields 11.714-121.600/m<sup>2</sup>; Shiponeni 2003) and fynbos (pristine vegetation 9.024/m<sup>2</sup>; Kaiser 2005). However, considerable lower values were found in pristine fynbos (1.100-1.900/m<sup>2</sup>, Holmes and Cowling 1997) and Chilean matoral (pristine vegetation 293-1.050/m<sup>2</sup>, abandoned fields 325-678/m<sup>2</sup>; Jiménez and Armesto 1992). Differences in seed abundance between soil layers are caused by seed migration in time (Baskin & Baskin; 1989). Once seeds have arrived in deeper soil layers, species have a higher potential persistence (Bakker *et al.* 1996). In general, more seedlings germinated from upper layers of the vertical soil profile, and these were

dominated by a few alien species, such as *Lolium multiflorum*. In turn, a high seed movement to deeper soil layers is present in most of the others species. Interestingly, little compositional differences were found, which is mirrored by findings of Walton (2006). An alarmingly high proportion of alien species is present in soil seed bank of abandoned fields (~50 %), pine plantation (~40 %), and pristine renosterveld (~30 %). These results need to be considered for management and restoration action.

#### *Combination of soil seed bank and vegetation data*

The combination of soil seed bank and vegetation data showed two contrasting results. On the one hand, renosterveld and pine plantation vegetation differed greatly from their beneath soil seed banks that is also mirrored by the low Sørensen Index. Low similarity of above- and below-ground vegetation is a well known phenomenon found in stable (Thompson 2000) and successional plant communities (Oosting and Humphreys 1940, Livingston and Allesio 1968, Brown and Oosterhuist 1981, Koniak and Everett 1982). Low species similarity is also reported from other vegetation types, depending on time since abandonment and management (Kalamees and Zobel 1998), and can increase with soil depth (Grandin and Rydin 1998, Wagner *et al.* 2003). Minor changes of soil seed bank was reported from grassland succession towards woodland (Milberg 1995). In this light, pine plantation seed bank could still be viable after anthropogenic land use change. On the other hand, vegetation and soil seed bank of abandoned fields was very similar indicated by the high Sørensen Index. That means that seed bank have derived mainly from the recent seed rain. Such pattern is also reported for abandoned fields in New York (Beatty 1991) and western Estonia (Kalamees and Zobel 1998). From the conservation point of view results indicate a low restoration potential for abandoned fields but a high recovery potential for pine plantation.

## Appendix 1

Species composition of soil seed bank. Persistence type (PT) following (Thompson *et al.* 1997): tra=transient, stp= short-term persistent, ltp = long-term persistent. Renosterveld (RV), Abandoned field (AF), Pine plantation (PP). Classification criteria based on presence/absence in vegetation (indicated by number) and soil seed bank (total number of emerged seedlings calculated as seedlings/m<sup>2</sup> with their depth distribution in brackets (upper layer 0-5 cm, lower layer 5-10 cm)). Bold seedling numbers show a species present in soil seed bank but absent in vegetation.

| Species  | Present in vegetation |      |      |      |      |      | Present in soil seed bank |                         |                         |                          |                          |                        |                         |
|--|-----------------------|------|------|------|------|------|---------------------------|-------------------------|-------------------------|--------------------------|--------------------------|------------------------|-------------------------|
|  | PT                    | AF1  | AF2  | PP   | RV1  | RV2  | RV3                       | AF1                     | AF2                     | PP                       | RV1                      | RV2                    | RV3                     |
| <i>Anthospermum hirtum</i> (Rubiaceae)                       | tra                   | -    | -    | -    | -    | 0.1  | 1.0                       | -                       | -                       | -                        | -                        | 720 (480, 240)         | 320 (240, 80)           |
| <i>Aspalathus flexuosa</i> (Fabaceae)                        | tra                   | -    | -    | -    | -    | 3.1  | 5.0                       | -                       | -                       | -                        | -                        | 320 (320, 0)           | 400 (400, 0)            |
| <i>Briza maxima</i> (Poaceae)                                | tra                   | -    | -    | 31.0 | -    | 10.6 | 11.2                      | -                       | -                       | 240 (240, 0)             | -                        | -                      | -                       |
| <i>Drosanthemum hispidum</i> (Mesembryanthemaceae)           | tra                   | -    | -    | 5.0  | -    | 0.6  | 0.4                       | -                       | -                       | -                        | -                        | 640 (400, 240)         | -                       |
| <i>Erodium malacoides</i> (Geraniaceae)                      | tra                   | 0.6  | 3.9  | -    | -    | 7.5  | 0.6                       | 400 (320, 80)           | 1200 (1040, 160)        | -                        | -                        | 1280 (1280, 0)         | -                       |
| <i>Erodium moschatum</i> (Geraniaceae)                       | tra                   | 0.7  | 0.6  | -    | -    | -    | -                         | -                       | 160 (160, 0)            | -                        | -                        | -                      | -                       |
| <i>Helichrysum pandurifolium</i> (Asteraceae)                | tra                   | 0.1  | 0.3  | 5.0  | 16.5 | 3.7  | 3.7                       | 1520 (1440, 80)         | 880 (720, 160)          | 5600 (4640, 960)         | 17360 (8720, 8640)       | 3440 (2080, 1360)      | 6320 (4000, 2320)       |
| <i>Otholobium hirtum</i> (Fabaceae)                          | tra                   | -    | 1.0  | -    | 1.8  | 3.1  | 2.0                       | -                       | 240 (0, 240)            | -                        | -                        | -                      | 240 (160, 80)           |
| <i>Rapistrum rugosum</i> (Brassicaceae)                      | tra                   | 0.5  | 1.9  | -    | -    | 2.2  | 5.0                       | 800 (560, 240)          | 480 (320, 160)          | -                        | 480 (240, 240)           | 560 (480, 80)          | -                       |
| <i>Selago corymbosa</i> (Scrophulariaceae)                   | tra                   | -    | -    | -    | 1.0  | -    | -                         | -                       | -                       | -                        | 29280 (18400, 10880)     | -                      | -                       |
| <i>Senecio hastatus</i> (Asteraceae)                         | tra                   | -    | -    | -    | 0.3  | 0.7  | 1.2                       | -                       | -                       | -                        | -                        | -                      | 80 (80, 0)              |
| <i>Tribolium uniolea</i> (Poaceae)                           | tra                   | -    | -    | 0.1  | -    | -    | 0.3                       | -                       | -                       | -                        | -                        | -                      | 160 (160, 0)            |
| <i>Aizoon sarmentosum</i> (Mesembryanthemaceae)              | stp                   | -    | -    | 5.0  | -    | -    | 0.3                       | -                       | -                       | 240 (0, 240)             | -                        | <b>80 (0, 80)</b>      | -                       |
| <i>Anagallis arvensis</i> ssp. <i>caerulea</i> (Primulaceae) | stp                   | -    | -    | -    | 11.1 | 9.6  | 4.3                       | <b>240 (80, 160)</b>    | <b>3760 (800, 2960)</b> | <b>2080 (1040, 1040)</b> | 46400(20800,25600)       | 9440 (3520, 5920)      | 6080 (2960, 3120)       |
| <i>Athanasia trifurcata</i> (Asteraceae)                     | stp                   | -    | -    | -    | 6.4  | -    | 1.5                       | -                       | -                       | <b>80 (80, 0)</b>        | 4400 (3760, 640)         | -                      | -                       |
| <i>Atriplex semibaccata</i> (Chenopodiaceae)                 | stp                   | -    | -    | 0.3  | -    | -    | -                         | -                       | <b>80 (0, 80)</b>       | -                        | -                        | <b>80 (80, 0)</b>      | -                       |
| <i>Avena barbata</i> (Poaceae)                               | stp                   | 40.3 | 35.3 | 6.7  | -    | 8.9  | -                         | 5680 (5120, 560)        | 12160 (11440, 720)      | 26160 (25840, 320)       | -                        | 5280 (4960, 320)       | <b>2800 (2800, 0)</b>   |
| <i>Briza minor</i> (Poaceae)                                 | stp                   | -    | -    | -    | 0.3  | -    | 2.3                       | -                       | <b>240 (160, 80)</b>    | -                        | 26000 (16480, 9520)      | <b>1600 (880, 720)</b> | 15920 (13280, 2640)     |
| <i>Bromus pectinatus</i> (Poaceae)                           | stp                   | 50.3 | 48.0 | -    | -    | 3.4  | 8.5                       | 640 (640, 0)            | 2400 (2000, 400)        | <b>2880 (2400, 480)</b>  | -                        | 320 (320, 0)           | 320 (320, 0)            |
| <i>Cerastium capense</i> (Caryophyllaceae)                   | stp                   | -    | -    | -    | -    | 0.6  | -                         | -                       | <b>160 (0, 160)</b>     | -                        | -                        | 5040 (4720, 320)       | -                       |
| <i>Diascia capensis</i> (Scrophulariaceae)                   | stp                   | -    | -    | 5.0  | -    | 0.1  | 0.6                       | -                       | -                       | 80 (80, 0)               | <b>80 (0, 80)</b>        | 1200 (800, 400)        | 320 (160, 160)          |
| <i>Didymodoxa capensis</i> (Urticaceae)                      | stp                   | -    | -    | -    | -    | 1.5  | -                         | -                       | -                       | <b>160 (160, 0)</b>      | <b>240 (160, 80)</b>     | 800 (400, 400)         | -                       |
| <i>Dimorphotheca pluvialis</i> (Asteraceae)                  | stp                   | -    | -    | 0.5  | -    | 0.7  | 0.6                       | -                       | -                       | -                        | -                        | <b>80 (80, 0)</b>      | -                       |
| <i>Echium plantagineum</i> (Boraginaceae)                    | stp                   | 7.8  | 25.3 | 5.0  | -    | -    | -                         | 2640 (1680, 960)        | 2800 (2240, 560)        | -                        | <b>80 (80, 0)</b>        | <b>480 (480, 0)</b>    | -                       |
| <i>Fumaria muralis</i> (Fumariaceae)                         | stp                   | -    | -    | -    | -    | 0.4  | 1.0                       | <b>960 (400, 560)</b>   | <b>80 (80, 0)</b>       | -                        | <b>2720 (1440, 1280)</b> | 160 (80, 80)           | 320 (240, 80)           |
| <i>Helichrysum cymosum</i> (Asteraceae)                      | stp                   | -    | -    | -    | 1.0  | -    | -                         | <b>80 (80, 0)</b>       | -                       | <b>80 (80, 0)</b>        | 21600 (8960, 12640)      | <b>160 (80, 80)</b>    | 160 (80, 80)            |
| <i>Helichrysum teretifolium</i> (Asteraceae)                 | stp                   | -    | -    | -    | -    | 0.3  | 1.7                       | <b>320 (320, 0)</b>     | <b>400 (240, 160)</b>   | <b>3120 (2560, 560)</b>  | <b>480 (480, 0)</b>      | 1600 (1280, 320)       | 12400 (8640, 3760)      |
| <i>Hermania alnifolia</i> (Malvaceae)                        | stp                   | -    | -    | -    | -    | -    | 0.3                       | -                       | -                       | -                        | -                        | <b>80 (80, 0)</b>      | 160 (160, 0)            |
| <i>Lolium multiflorum</i> (Poaceae)                          | stp                   | 43.3 | 19.6 | -    | -    | -    | -                         | 44800 (42080, 2720)     | 82400 (75360, 7040)     | <b>800 (720, 80)</b>     | <b>160 (160, 0)</b>      | <b>880 (880, 0)</b>    | <b>9040 (8640, 400)</b> |
| <i>Medicago polymorpha</i> (Fabaceae)                        | stp                   | 3.4  | 4.3  | -    | -    | -    | -                         | 80 (80, 0)              | 480 (480, 0)            | <b>80 (0, 80)</b>        | -                        | -                      | <b>80 (80, 0)</b>       |
| <i>Oxalis compressa</i> (Oxalidaceae)                        | stp                   | -    | -    | -    | -    | 10.5 | -                         | <b>3280 (2880, 400)</b> | <b>2800 (2000, 800)</b> | -                        | <b>400 (160, 240)</b>    | 240 (80, 160)          | <b>800 (640, 160)</b>   |
| <i>Phalaris minor</i> (Poaceae)                              | stp                   | -    | -    | -    | -    | 1.8  | -                         | <b>80 (0, 80)</b>       | -                       | <b>80 (80, 0)</b>        | -                        | 160 (160, 0)           | <b>80 (80, 0)</b>       |
| <i>Picris echioides</i> (Asteraceae)                         | stp                   | 0.1  | 0.1  | -    | -    | -    | -                         | 2800 (2080, 720)        | 400 (320, 80)           | <b>160 (0, 160)</b>      | <b>80 (80, 0)</b>        | -                      | -                       |
| <i>Pseudognaphalium undulatum</i> (Asteraceae)               | stp                   | -    | -    | 0.3  | -    | -    | -                         | <b>240 (160, 80)</b>    | <b>800 (640, 160)</b>   | 480 (320, 160)           | <b>400 (400, 0)</b>      | -                      | <b>320 (160, 160)</b>   |
| <i>Sebaea aurea</i> (Gentianaceae)                           | stp                   | -    | -    | 5.0  | -    | -    | 0.3                       | -                       | -                       | -                        | -                        | <b>240 (160, 80)</b>   | 1520 (960, 560)         |
| <i>Senecio pubigerens</i> (Asteraceae)                       | stp                   | -    | -    | -    | -    | -    | 5.0                       | <b>240 (0, 240)</b>     | <b>160 (80, 80)</b>     | <b>160 (80, 80)</b>      | <b>720 (480, 240)</b>    | <b>240 (0, 240)</b>    | 560 (160, 400)          |
| <i>Solanum guineense</i> (Solanaceae)                        | stp                   | -    | -    | 51.0 | 0.3  | 0.1  | 0.3                       | <b>80 (80, 0)</b>       | -                       | -                        | 240 (160, 80)            | -                      | 80 (80, 0)              |
| <i>Sonchus asper</i> (Asteraceae)                            | stp                   | -    | -    | -    | -    | 0.5  | -                         | <b>80 (80, 0)</b>       | -                       | <b>320 (320, 0)</b>      | -                        | 320 (320, 0)           | 80 (0, 80)              |
| <i>Sonchus oleraceae</i> (Asteraceae)                        | stp                   | -    | 0.3  | -    | -    | -    | -                         | -                       | 160 (160, 0)            | <b>400 (400, 0)</b>      | <b>480 (240, 240)</b>    | -                      | <b>960 (480, 480)</b>   |
| <i>Spiloxene flaccida</i> (Hypoxidaceae)                     | stp                   | -    | -    | 5.0  | -    | -    | 1.0                       | -                       | <b>80 (80, 0)</b>       | -                        | -                        | -                      | -                       |
| <i>Stachys aethiopica</i> (Lamiaceae)                        | stp                   | -    | -    | 0.7  | -    | 6.7  | 0.3                       | -                       | <b>80 (80, 0)</b>       | 240 (160, 80)            | -                        | 960 (400, 560)         | 80 (80, 0)              |
| <i>Sutera uncinata</i> (Scrophulariaceae)                    | stp                   | -    | -    | -    | -    | 5.0  | 0.3                       | -                       | -                       | <b>320 (160, 160)</b>    | <b>1840 (1200, 640)</b>  | 1920 (1600, 320)       | 1280 (1120, 160)        |

## Appendix 1 cont.

|  |     |      |      |     |     |      |                   |                  |                 |                |                  |                   |                |
|--|-----|------|------|-----|-----|------|-------------------|------------------|-----------------|----------------|------------------|-------------------|----------------|
| <i>Acacia saligna</i> (Fabaceae)                               | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | 80 (80, 0)     | -                | -                 | -              |
| <i>Aira cupaniana</i> (Poaceae)                                | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (80, 0)      | -              | 320 (0, 320)     | -                 | -              |
| <i>Anagallis arvensis</i> subsp. <i>arvensis</i> (Primulaceae) | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 160 (80, 80)     | -                 | -              |
| <i>Conyza scabriflora</i> (Asteraceae)                         | ltp | -    | -    | -   | -   | -    | 320 (160, 160)    | -                | 400 (320, 80)   | 160 (0, 160)   | 240 (240, 0)     | 240 (240, 0)      | -              |
| <i>Cotula turbinata</i> (Asteraceae)                           | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (80, 0)      | -              | -                | -                 | -              |
| <i>Crassula ciliata</i> (Crassulaceae)                         | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 160 (160, 0)     | -                 | -              |
| <i>Cyanella lutea</i> (Iridaceae)                              | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 80 (80, 0)       | -                 | -              |
| <i>Ehrharta longiflora</i> (Poaceae)                           | ltp | -    | -    | -   | -   | -    | 80 (80, 0)        | -                | 80 (80, 0)      | -              | -                | 160 (160, 0)      | -              |
| <i>Gastidium phleoides</i> (Poaceae)                           | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (80, 0)      | -              | -                | -                 | -              |
| geophyte sp.01   | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 80 (0, 80)       | -                 | -              |
| geophyte sp.04   | ltp | -    | -    | -   | -   | -    | 240 (0, 240)      | 240 (80, 160)    | 1600 (880, 720) | 320 (0, 320)   | 160 (80, 80)     | 1440 (1440, 0)    | -              |
| <i>Gnidia laxa</i> (Thymelaeaceae)                             | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (0, 80)      | -              | -                | -                 | -              |
| grass sp.01 (Poaceae)  | ltp | -    | -    | -   | -   | -    | -                 | 80 (80, 0)       | -               | -              | 240 (240, 0)     | -                 | -              |
| <i>Helichrysum asperum</i> (Asteraceae)                        | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (80, 0)      | -              | 80 (80, 0)       | -                 | -              |
| <i>Helichrysum indicum</i> (Asteraceae)                        | ltp | -    | -    | -   | -   | -    | -                 | 80 (80, 0)       | -               | -              | -                | 560 (480, 80)     | -              |
| <i>Helichrysum luteo-album</i> (Asteraceae)                    | ltp | -    | -    | -   | -   | -    | 80 (0, 80)        | 400 (320, 80)    | 80 (80, 0)      | 80 (80, 0)     | 80 (0, 80)       | 320 (320, 0)      | -              |
| herb sp.02   | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 160 (0, 160)     | -                 | -              |
| herb sp.03   | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | 240 (240, 0)   | -                | -                 | -              |
| herb sp.07   | ltp | -    | -    | -   | -   | -    | 80 (80, 0)        | -                | -               | -              | -                | -                 | -              |
| herb sp.08   | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 80 (0, 80)       | 480 (480, 0)      | -              |
| herb sp.10   | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 160 (160, 0)     | -                 | -              |
| herb sp.11   | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 80 (0, 80)       | -                 | -              |
| herb sp.13   | ltp | -    | -    | -   | -   | -    | -                 | 80 (80, 0)       | -               | -              | -                | -                 | -              |
| herb sp.14   | ltp | -    | -    | -   | -   | -    | -                 | 80 (80, 0)       | -               | 80 (0, 80)     | -                | -                 | -              |
| <i>Hypericum perforatum</i> (Clusiaceae)                       | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (0, 80)      | -              | -                | -                 | -              |
| <i>Isolepis marginata</i> (Cyperaceae)                         | ltp | -    | -    | -   | -   | -    | -                 | 800 (240, 560)   | -               | 880 (480, 400) | 160 (160, 0)     | 2720 (1440, 1280) | -              |
| <i>Kickxia spuria</i> (Scrophulariaceae)                       | ltp | -    | -    | -   | -   | -    | 4000 (1840, 2160) | 880 (720, 160)   | -               | -              | -                | -                 | -              |
| <i>Lampranthus peacockiae</i> (Mesembryanthemaceae)            | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 160 (0, 160)     | -                 | -              |
| <i>Lampranthus</i> sp. (Mesembryanthemaceae)                   | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (80, 0)      | -              | -                | -                 | -              |
| <i>Lobelia erinus</i> (Campanulaceae)                          | ltp | -    | -    | -   | -   | -    | -                 | 80 (80, 0)       | 240 (0, 240)    | 160 (80, 80)   | -                | 1680 (800, 880)   | -              |
| <i>Lythrum hyssopifolia</i> (Lythraceae)                       | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | 240 (240, 0)   | -                | -                 | -              |
| <i>Monopsis</i> sp. (Campanulaceae)                            | ltp | -    | -    | -   | -   | -    | 80 (0, 80)        | -                | -               | -              | -                | -                 | -              |
| <i>Nidorella foetida</i> (Asteraceae)                          | ltp | -    | -    | -   | -   | -    | -                 | -                | 400 (400, 0)    | -              | 80 (80, 0)       | -                 | -              |
| <i>Ornithogalum graminifolium</i> (Hyacinthaceae)              | ltp | -    | -    | -   | -   | -    | -                 | -                | 320 (240, 80)   | -              | -                | -                 | -              |
| <i>Oxalis pes-caprae</i> (Oxalidaceae)                         | ltp | 13.8 | 12.5 | 2.2 | 8.4 | 12.3 | 9.7               | 160 (160, 0)     | 320 (160, 160)  | 240 (240, 0)   | 80 (80, 0)       | 240 (160, 80)     | 240 (240, 0)   |
| <i>Oxalis</i> sp. (Oxalidaceae)                                | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 80 (0, 80)       | 160 (80, 80)      | -              |
| <i>Pelargonium</i> sp. (Geraniaceae)                           | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 80 (0, 80)       | -                 | -              |
| <i>Polygonum aviculare</i> (Polygonaceae)                      | ltp | -    | -    | -   | -   | -    | 480 (240, 240)    | 80 (0, 80)       | -               | -              | -                | -                 | -              |
| <i>Pterygodium catholicum</i> (Orchidaceae)                    | ltp | -    | -    | 5.0 | 9.9 | -    | 0.7               | -                | -               | 80 (80, 0)     | -                | -                 | -              |
| <i>Rhus</i> sp. (Anacardiaceae)                                | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (80, 0)      | -              | -                | -                 | -              |
| <i>Roellia ciliata</i> (Campanulaceae)                         | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | -                | 80 (0, 80)        | -              |
| <i>Rumex acetosella</i> (Polygonaceae)                         | ltp | -    | -    | -   | -   | -    | -                 | 80 (80, 0)       | -               | -              | -                | -                 | -              |
| <i>Senecio burchelli</i> (Asteraceae)                          | ltp | -    | -    | -   | -   | -    | -                 | 1280 (1040, 240) | -               | -              | -                | -                 | 720 (480, 240) |
| <i>Senecio elegans</i> (Asteraceae)                            | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (80, 0)      | -              | -                | -                 | -              |
| <i>Senecio pierophorus</i> (Asteraceae)                        | ltp | -    | -    | -   | -   | -    | -                 | 160 (160, 0)     | 80 (80, 0)      | 80 (80, 0)     | 80 (80, 0)       | 160 (160, 0)      | -              |
| <i>Senecio vernalis</i> (Asteraceae)                           | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 320 (320, 0)     | -                 | -              |
| <i>Silene cretica</i> (Caryophyllaceae)                        | ltp | -    | -    | -   | -   | -    | -                 | -                | 80 (0, 80)      | -              | 2160 (1920, 240) | -                 | -              |
| <i>Stellaria media</i> (Caryophyllaceae)                       | ltp | -    | -    | -   | -   | -    | 320 (240, 80)     | -                | -               | 80 (0, 80)     | 6400 (5680, 720) | -                 | -              |
| <i>Tribolium hispidum</i> (Poaceae)                            | ltp | -    | -    | -   | -   | -    | -                 | -                | -               | -              | 80 (80, 0)       | 80 (80, 0)        | -              |

## Appendix 2

List of species present in vegetation and absent in soil seed bank. Site occurrences are given in brackets. Renosterveld (RV), Abandoned field (AF), Pine plantation (PP).

### Identified species:

|  |  |   |
|--|--|---|
| <i>Anthospermum spathulatum</i> (RV1,2,3)  | <i>Galium capense</i> (RV1)            | <i>Pelargonium lobatum</i> (RV2)              |
| <i>Arcotheca calendula</i> (PP)            | <i>Geizorhiza asper</i> (RV2)          | <i>Pelargonium myrrhifolium</i> (RV3)         |
| <i>Arctopus echinatus</i> (RV2,3)          | <i>Geranium canescens</i> (AF2, RV3)   | <i>Pelargonium</i> sp.2 (PP)                  |
| <i>Asparagus asparagoides</i> (RV2)        | <i>Geranium molle</i> (RV2,3)          | <i>Plantago lanceolata</i> (PP)               |
| <i>Asparagus capensis</i> (PP)             | <i>Gymnosporia buxifolia</i> (PP, RV3) | <i>Podalyria sericia</i> (RV2)                |
| <i>Asparagus</i> sp. (RV3)                 | <i>Hebenstreita repens</i> (RV2)       | <i>Pterona hirsuta</i> (RV2,3)                |
| <i>Asphalathus acanthophylla</i> (RV3)     | <i>Helichrysum revolutum</i> (RV1,2,3) | <i>Raphanus raphanistrum</i> (AF1,2)          |
| <i>Babiana stricta</i> (PP, RV2)           | <i>Helichrysum</i> sp.1 (RV2)          | <i>Reseda lutea</i> (RV3)                     |
| <i>Berkheya armata</i> (PP, RV3)           | <i>Hemimeris racemosa</i> (RV2,3)      | <i>Rhus laevigata</i> (PP, RV1,2,3)           |
| <i>Berkheya rigida</i> (PP, RV1,2,3)       | <i>Hermannia rugosa</i> (RV2)          | <i>Rhus tomentosa</i> (RV1,3)                 |
| <i>Bobartia indica</i> (RV2)               | <i>Hesperantha falcata</i> (PP, RV3)   | <i>Romulea</i> sp.1 (RV3)                     |
| <i>Bulbine</i> sp. (PP)                    | <i>Hesperantha radiata</i> (RV3)       | <i>Rumex cordatus</i> (RV1,2)                 |
| <i>Bulbinella triquetra</i> (PP, RV3)      | <i>Indigofera</i> sp. (RV3)            | <i>Salvia africana-caerulea</i> (PP, RV1,2,3) |
| <i>Carpobrotus acinaciformis</i> (RV2)     | <i>Lachenalia longibracteata</i> (RV2) | <i>Salvia africana-lutea</i> (RV3)            |
| <i>Chasmanthe floribunda</i> (RV1)         | <i>Lampranthus emarginatus</i> (RV3)   | <i>Satyrium odorum</i> (RV3)                  |
| <i>Cheilanthes capensis</i> (RV1,2,3)      | <i>Leysera gnaphalodes</i> (RV2,3)     | <i>Scabiosa columbaria</i> (RV3)              |
| <i>Chlorophytum undulatum</i> (RV2)        | <i>Lotononis prostrata</i> (RV3)       | <i>Senecio rosmarinifolius</i> (RV3)          |
| <i>Cissampelos capensis</i> (RV1,2,3)      | <i>Lupinus angustifolius</i> (AF1,2)   | <i>Senecio</i> sp.1 (RV2)                     |
| <i>Crassula capensis</i> (RV1)             | <i>Lycium afrum</i> (PP, RV3)          | <i>Senecio</i> sp.2 (RV2)                     |
| <i>Cyphia digitata</i> (RV2,3)             | <i>Montinia caryophyllaceae</i> (RV3)  | <i>Silene undulata</i> (PP, RV2)              |
| <i>Cyphia phytouma</i> (PP)                | <i>Moraea</i> sp. (RV3)                | <i>Solanum</i> sp.1 (RV1)                     |
| <i>Cytinus sanguineus</i> (RV2)            | <i>Moraea</i> sp.2 (AF1,2, PP)         | <i>Sparaxis villosa</i> (PP, RV3)             |
| <i>Dolichos decumbens</i> (PP)             | <i>Moraea miniata</i> (PP, RV2)        | <i>Spiloxene capensis</i> (PP, RV2)           |
| <i>Ehrharta melicoides</i> (RV1,2)         | <i>Muraltia ononidifolia</i> (PP, RV2) | <i>Tetragonia spicata</i> (AF2, PP, RV2,3)    |
| <i>Elytropappus rhinocerotis</i> (RV1,2,3) | <i>Nemesia barbata</i> (RV3)           | <i>Themeda triandra</i> (RV3)                 |
| <i>Empodium plicatum</i> (RV3)             | <i>Olea europaea</i> (PP, RV1)         | <i>Torilles arvensis</i> (RV1,2,3)            |
| <i>Erhartha longiflora</i> (PP)            | <i>Ornithogalum thyrsoides</i> (PP)    | <i>Trachyandra hirsutiflora</i> (PP)          |
| <i>Erharta calycina</i> (PP, RV2,3)        | <i>Osteospermum spinosum</i> (PP, RV3) | <i>Trachyandra muricata</i> (PP, RV2,3)       |
| <i>Eriocephalus africanus</i> (RV2,3)      | <i>Othonna arborescens</i> (RV2)       | <i>Tulbaghia capensis</i> (PP, RV3)           |
| <i>Euphorbia burmanii</i> (RV2)            | <i>Othonna ciliata</i> (RV2)           | <i>Vicia sativa</i> (PP)                      |
| <i>Euphorbia genistoides</i> (PP, RV1,3)   | <i>Oxalis argyrophylla</i> (RV1)       | <i>Viscum capense</i> (RV1, RV3)              |
| <i>Euphorbia helioscopia</i> (AF2)         | <i>Oxalis lanata</i> (PP, RV1)         | <i>Zantedeschia aethopica</i> (RV1,3)         |
| <i>Felicia dubia</i> (RV2)                 | <i>Oxalis obtusa</i> (RV2)             |   |
| <i>Felicia fruticosa</i> (RV3)             | <i>Oxalis tomentosa</i> (PP, RV2)      |   |
| <i>Festuca glabra</i> (PP, RV2)            |  |   |

### Unidentified geophytes:

geophyte 01 (AF1,2), geophyte 02 (PP), geophyte 03 (PP), geophyte 04 (RV2), geophyte 05 (RV2), geophyte 06 (RV3), geophyte 07 (RV3), geophyte 08 (RV3), geophyte 09 (RV3), geophyte 10 (RV3), geophyte 11 (RV3), geophyte 12 (RV3), geophyte 13 (RV3)

### Unidentified grasses:

grass 02 (RV1)

### Unidentified herbs:

herb 01 (PP), herb 02 (RV1), herb 03 (RV3)

### Unidentified shrubs:

shrub 01 (AF2), shrub 02 (PP), shrub 04 (PP, RV3), shrub 05 (RV3), shrub 06 (RV3)

### **3 Rise like a phoenix? Insights from smoke-primer experiments on pristine and degraded renosterveld soil seed bank.**

#### **Abstract**

Fire and smoke compounds are known to have a germination enhancing effect. Besides that, fire is a sound management tool in fire prone ecosystems, such as well studied fynbos vegetation of South Africa. However, little experimental data exist on fire response in renosterveld, once the second largest vegetation type in the Cape Floristic Region. Agriculture, alien species and urban development resulted in extremely high habitat loss and fragmentation of West Coast Centre renosterveld, leaving ninety percent in a degraded state. The Tygerberg Nature Reserve is one of largest remaining renosterveld fragment in the Cape lowlands. Here, the study examined the influence of smoke-primer (i.e. fire surrogate) on soil seed bank of three pristine and adjacent degraded renosterveld sites in order to evaluate fire as a management tool for renosterveld. Although some species were dependent on smoke-primer application, this treatment only had a significant effect on 13 plant species. Furthermore, a detrended component analysis of both treatments revealed little differences. Nevertheless, significantly more species and seed numbers occurred at some renosterveld sites. Results indicate that fire should be used in renosterveld management in order to secure the survival of rare species with low abundances. In a more general context, the low fire response might indicate that renosterveld could be largely a disturbance adapted vegetation and less fire-prone than fynbos. Caution is needed if fire application should be used in restoration. Fire treatment seems to be not appropriate for abandoned fields with *Echium plantagineum* infestation, but was of little concern in pine plantation.

## Introduction

A fire-free planet would look very differently because fire impact on vegetation is similar to an unselective mega herbivore, thereby shaping entire floras (Bond *et al.* 2005). Mediterranean-type ecosystems are prime examples for fire adapted vegetation (Cowling *et al.* 1996). Particular traits such as serotiny (Lamont and Enright 2000, Schwilk and Ackerly 2001) and myrmecochory (Giladi 2006) are seen as response to regular fire events (Cowling 1992). It is interesting that fire stimulus can also apply in non-fire prone environments (Pierce *et al.* 1995). Fire enhances germination indirectly by smoke extracts (De Lange and Boucher 1990, Brown 1993, Brown *et al.* 1993, Baxter *et al.* 1994, Van Staden *et al.* 2000, Light *et al.* 2009). Only recently, karrikinolide, a butenolide compound isolated from smoke, was identified as an active germination inducing substance (Flematti *et al.* 2004, Van Staden *et al.* 2004, Dixon *et al.* 2009, Nelson *et al.* 2009).

A large proportion of the species-rich Cape Floristic Region was once covered with fire-prone shrublands, namely, fynbos found on nutrient-poor soils and renosterveld occurring on fertile soils (Rebelo *et al.* 2006). In contrast to mountain areas, the lowland shrublands have suffered severe transformation and habitat loss due to agriculture, urban development and alien invasive plant species, especially in the last century.

Fire is used as an important tool to manage pristine and to restore degraded habitats. However, studies have mainly focused on fynbos vegetation and restoring from *Acacia* sp. invasion (Holmes *et al.* 2000, Holmes 2002, Cilliers *et al.* 2004, van Wilgen 2009). Soil seed banks in fynbos respond greatly to fire (Kaiser 2005). Renosterveld research has started to aim a better understanding of fire response and its restoration potential (Musil *et al.* 2005, Memiaghe 2008).

The study system presented here is West Coast Centre renosterveld (Rebelo *et al.* 2006) that had a long history as pasture ground for KhoiKhoi pastoralists (Schweitzer and Scott 1973, Klein 1986). However, tremendous land use change and subsequent habitat loss followed the European arrival (Newton 2008). The suitability of renosterveld for dry land agriculture have left only 10 % of its former extent (von Hase *et al.* 2003a). The remaining renosterveld fragments have a poor conservation status and are highly threatened (Cowling *et al.* 1999b). Abandonment of agricultural areas began in the 1980s and those are infested with European pasture grasses. Abandoned fields or pine plantations cover one percent of former renosterveld (von Hase *et al.* 2003b) and are often the only potential restoration option in order to increase renosterveld areas (Krug and Krug 2007).

Initial soil seed bank studies in renosterveld concentrated on edge effects of fragments (Shiponeni 2003, Muhl 2008) or the restoration potential of abandoned fields in mountain renosterveld (Saayman and Botha 2008). Although fire response were studied in renosterveld (Midoko-Iponga 2004, Musil *et al.* 2005, Memiaghe 2008), no observation of seed bank took place.

The main objective of this study was to fill this particular knowledge gap and to describe the influence of smoke-primer as a fire surrogate on i) soil seed bank of three sites with pristine renosterveld vegetation and ii) as a management tool for adjacent degraded habitats (i.e. two abandoned fields and one pine plantation). The study was realized at Tygerberg Nature Reserve, the largest remaining renosterveld fragment in the Cape region, north of Cape Town. Specifically, it was asked if smoke primer application can enhance seed germination of indigenous renosterveld species and therefore can be considered as a management tool for degraded sites.

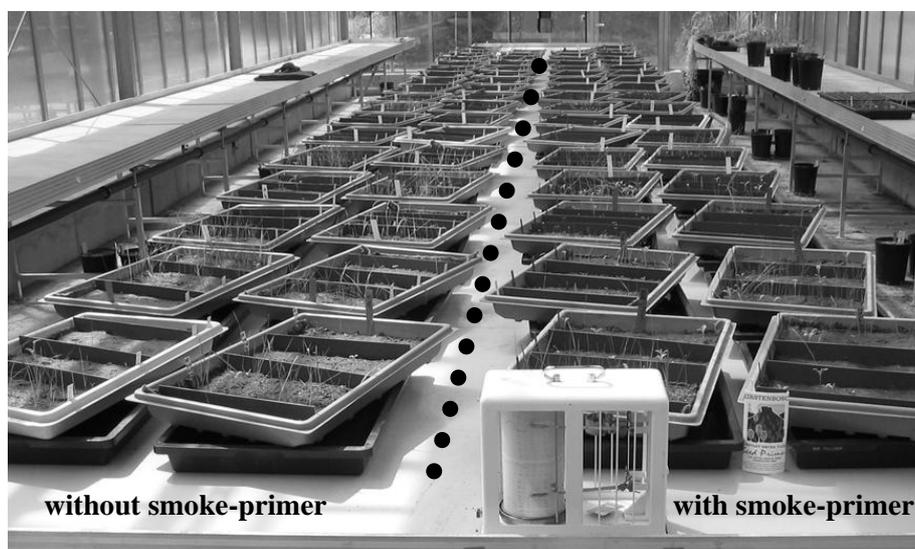
## Material and Methods

### *Study area and sites*

Information about the study area and sites are mentioned in chapter two, page 20.

### *Soil seed bank sampling, germination experiment and data analysis*

Information is given in chapter two, page 21-22. Additionally to the treatment described in chapter two, twice the amount of soil samples was used (2 x 62.5 cm<sup>3</sup>) and watered either without or with smoke-primer, respectively (Fig. 9). The smoke-primer was obtained from Kirstenbosch Botanical Garden (Kirstenbosch Instant Smoke Plus).



**Fig. 9.** Two rows of soil samples without and with smoke-primer treatment.

## Results

During the germination experiment 6405 seedlings emerged from the soil seed bank without smoke-primer treatment, whereas 8159 seedlings were counted with smoke-primer treatment (Table 4). Smoke-primer treatment was followed by significant higher species number in RV3 and higher seed numbers occurred in RV1, RV3 (total and upper soil layer), and for RV2, RV3 (lower soil layer). No effect could be detected for abandoned fields or pine plantation. Thirteen species showed a significant increase germination response after smoke-primer treatment (Table 5), one of them was a Red Data List species (*Aspalathus flexuosa*). Low clustering of smoke-primer treatments was visible (Fig. 10). Renosterveld vegetation shows little grouping whereas abandoned fields are more clustered. Pine plantation shows an intermediate position between both.

**Table 4. Soil seed bank data with and without smoke-primer treatment.**

Renosterveld (RV), abandoned field (AF), pine plantation (PP). Means and standard error of means are given. Significant differences of ANOVA-analysis with LSD post-hoc test between sites (n=10) are indicated with asterisk and different letters. Bold numbers show significant higher values. Sørensen index is given as similarity measure. Soil horizon comparison was done via joined *t*-test. Significance level  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)

| Treatments:                                    |      |                                 |                                |                                |                           |                           |                          |  |
|--|------|---------------------------------|--------------------------------|--------------------------------|---------------------------|---------------------------|--------------------------|--|
| Without smoke-primer (W)                       |      |                                 |                                |                                |                           |                           |                          |  |
| Smoke-primer treatment (S)                     |      |                                 |                                |                                |                           |                           |                          |  |
| Comparison (C)                                 |      | RV1                             | RV2                            | RV3                            | AF1                       | AF2                       | PP                       |  |
| Total no. of species                           | W    | 37                              | 50                             | 40                             | 32                        | 37                        | 39                       |  |
|  | S    | 39                              | 55                             | 55                             | 32                        | 43                        | 43                       |  |
| Mean no. of species per sample (0-10cm)        | W*** | 19.7 ± 0.76 <sup>a</sup>        | 18.0 ± 1.67 <sup>a</sup>       | 19.0 ± 1.00 <sup>a</sup>       | 14.8 ± 1.23 <sup>b</sup>  | 15.2 ± 1.36 <sup>ab</sup> | 13.9 ± 1.13 <sup>b</sup> |  |
|  | S*** | 22.4 ± 1.12 <sup>a</sup>        | 23.0 ± 2.25 <sup>a</sup>       | <b>23.2 ± 1.17<sup>a</sup></b> | 15.6 ± 1.33 <sup>b</sup>  | 17.6 ± 1.34 <sup>b</sup>  | 14.6 ± 0.92 <sup>b</sup> |  |
|  | C    | n.s                             | n.s                            | *                              | n.s                       | n.s                       | n.s                      |  |
| Total no. of seeds                             | W    | 1962                            | 621                            | 872                            | 904                       | 1447                      | 599                      |  |
|  | S    | 2582                            | 890                            | 1496                           | 882                       | 1652                      | 657                      |  |
| Total no. of seeds per 1m <sup>2</sup>         | W*** | 15634 ± 1471 <sup>c</sup>       | 4948 ± 1398 <sup>a</sup>       | 6948 ± 795 <sup>a</sup>        | 7203 ± 953 <sup>a</sup>   | 11530 ± 1727 <sup>b</sup> | 4773 ± 821 <sup>a</sup>  |  |
|  | S*** | <b>20574 ± 2276<sup>c</sup></b> | 7091 ± 1424 <sup>b</sup>       | <b>11928 ± 976<sup>a</sup></b> | 7028 ± 1203 <sup>b</sup>  | 13163 ± 1695 <sup>a</sup> | 5235 ± 921 <sup>b</sup>  |  |
|  | C    | ***                             | n.s                            | ***                            | n.s                       | n.s                       | n.s                      |  |
| Mean no. of seeds per 1m <sup>2</sup> (0-5cm)  | W*** | 8383 ± 871 <sup>bc</sup>        | 3578 ± 1092 <sup>a</sup>       | 5219 ± 796 <sup>a</sup>        | 6191 ± 934 <sup>ab</sup>  | 10016 ± 1512 <sup>c</sup> | 4199 ± 791 <sup>a</sup>  |  |
|  | S*** | <b>11649 ± 1309<sup>b</sup></b> | 5195 ± 1247 <sup>a</sup>       | <b>8796 ± 1030<sup>b</sup></b> | 5888 ± 1100 <sup>ab</sup> | 11378 ± 1558 <sup>b</sup> | 4486 ± 938 <sup>a</sup>  |  |
|  | C    | ***                             | n.s                            | ***                            | n.s                       | n.s                       | n.s                      |  |
| Mean no. of seeds per 1m <sup>2</sup> (5-10cm) | W*** | 7251 ± 770 <sup>c</sup>         | 1371 ± 361 <sup>ab</sup>       | 1729 ± 336 <sup>ab</sup>       | 1012 ± 232 <sup>ab</sup>  | 1514 ± 351 <sup>ab</sup>  | 574 ± 80 <sup>a</sup>    |  |
|  | S*** | 8924 ± 1179 <sup>c</sup>        | <b>1896 ± 378<sup>ab</sup></b> | <b>3132 ± 410<sup>b</sup></b>  | 1139 ± 223 <sup>a</sup>   | 1785 ± 280 <sup>ab</sup>  | 749 ± 77 <sup>a</sup>    |  |
|  | C    | n.s                             | *                              | **                             | n.s                       | n.s                       | n.s                      |  |
| Mean no. of seeds (0-5cm vs. 5-10cm)           | W    | n.s                             | **                             | **                             | ***                       | ***                       | **                       |  |
|  | S    | n.s                             | *                              | **                             | ***                       | ***                       | **                       |  |
| Sørensen index                                 | C    | 0.81                            | 0.51                           | 0.51                           | 0.52                      | 0.49                      | 0.69                     |  |

**Table 5. Species with significant increased germination response after smoke-primer treatment.**

Renosterveld (RV), abandoned field (AF), pine plantation (PP). Significant Z-values derived from Wilcoxon test (\*=p<0.05, \*\*=p<0.01, \*\*\*=p<0.001) and shown bold. Species present in soil seed bank, without significant smoke primer response (P), Species not present in soil seed bank (-).

|                                  | RV1            | RV2           | RV3            | AF1           | AF2            | PP            |
|----------------------------------|----------------|---------------|----------------|---------------|----------------|---------------|
| <b>Renosterveld species</b>      |                |               |                |               |                |               |
| <i>Aspalathus flexuosa</i>       | P              | P             | P              | -             | <b>-2,5 *</b>  | -             |
| <i>Conyza scabrida</i>           | <b>-2,6 *</b>  | P             | P              | <b>-2,0 *</b> | -              | P             |
| <i>Drosanthemum hispidum</i>     | P              | <b>-2,5 *</b> | <b>-2,2 *</b>  | -             | -              | P             |
| <i>Helichrysum pandurifolium</i> | <b>-2,2 *</b>  | P             | <b>-2,1 *</b>  | <b>-2,2 *</b> | P              | P             |
| <i>Helichrysum teretifolium</i>  | P              | P             | <b>-2,8 **</b> | P             | P              | P             |
| <i>Isolepis marginata</i>        | <b>-2,7 **</b> | P             | P              | P             | -              | P             |
| <i>Oxalis compressa</i>          | P              | P             | P              | P             | <b>-2,2 *</b>  | <b>-2,1 *</b> |
| <i>Selago corymbosa</i>          | <b>-2,1 *</b>  | -             | P              | -             | -              | -             |
| <i>Senecio pubigerens</i>        | P              | P             | <b>-2,2 *</b>  | P             | P              | P             |
| <i>Sutera uncinata</i>           | P              | <b>-2,4 *</b> | P              | -             | P              | P             |
| <b>Alien species</b>             |                |               |                |               |                |               |
| <i>Anagallis arvensis</i>        | P              | P             | P              | P             | <b>-2,2 *</b>  | P             |
| <i>Echium plantagineum</i>       | P              | P             | P              | <b>-2,5 *</b> | <b>-2,8 **</b> | -             |
| <i>Fumaria muralis</i>           | P              | P             | P              | <b>-2,1 *</b> | P              | -             |

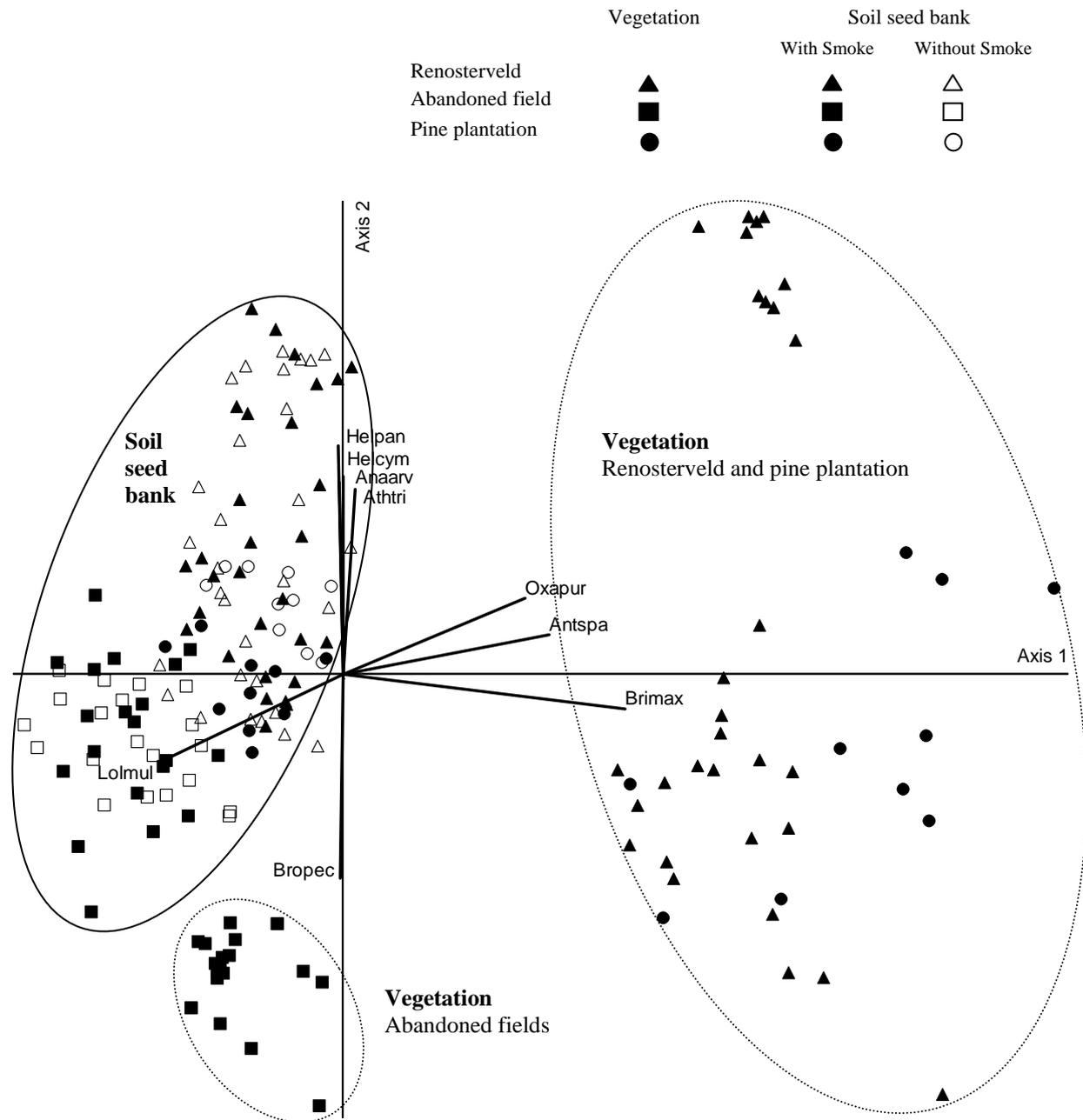
Species that occurred only after smoke primer treatment in low abundances with no statistical significance:

Identified species:

*Anisodentea biflora* (Malvac.), *Antimima aristulata* (Mesembryanthemac.), *Arcotheca calendula* (Asterac.), *Carpobrotus* sp. (Mesembryanthemac.), *Felicia dubia* (Asterac.), *Hesperantha radiata* (Iridac.), *Juncus capensis* (Juncac.), *Oxalis purpurea* (Oxalidac.), *Pinus radiata* (Pineac.), *Plantago lanceolata* (Plantaginac.), *Rhus laevigata* (Anacardiaceae), *Senecio scapiflorus* (Asterac.), *Sutera hispida* (Scrophulariac.), *Sutera* sp. (Scrophulariac.), *Torilis arvensis* (Apiac.), *Wahlenbergia* sp. (Campanulac.), *Wahlenbergia capensis* (Campanulac.).

Unidentified species:

Geophyte 2,3; herb 1,4,5,6,9,12.



**Fig. 10. DCA of vegetation and soil seed bank with and without smoke-primer treatment.**

Vegetation (dotted line), soil seed bank (white, full line) and soil seed bank with smoke-primer treatment (dark, full line). Renosterveld (triangles), pine plantation (circles), abandoned fields (squares). Power-transformed matrix of 232 taxa and 180 samples. Increment axis 1: 0.33, Axis 2: 0.23. Length of gradient 4.1.  $r^2$ -cut off: 0.35. Species correlated with axis 1: *Briza maxima* ( $r^2=0.55$ ), *Anthospermum spathulum* ( $r^2=0.40$ ), *Oxalis purpurea* ( $r^2=0.35$ ), *Lolium multiflorum* ( $r^2=0.36$ ), species correlated with axis 2: *Helichrysum pandurifolium* (0.45), *Anagallis arvensis* ( $r^2=0.39$ ), *Helichrysum cymosum* ( $r^2=0.38$ ), *Athenisia trifurcata* ( $r^2=0.36$ ), *Bromus pectinatus* ( $r^2=0.41$ ).

## Discussion

Although more seeds appeared after smoke-treatment, this was only significant for certain renosterveld sites. Species-wise, smoke application was only significant in one renosterveld site. Thirteen species, three of them alien, showed a significant higher germination response

after smoke-primer treatment. Although on a non-significant level, twenty-six species appeared only after smoke-primer treatment. Interestingly for fire-prone vegetation, smoke response in this study is low compared to fynbos vegetation. Smoke-induced differences in this vegetation type normally occur at much higher magnitude (with/without smoke: 7.040/1.984 seeds/m<sup>2</sup>, Kaiser 2005). For fynbos species, smoke extract is a germination cue *per se* (Light *et al.* 2009) and due to smoke-derived butenolide (Flematti *et al.* 2004) that has similarity to germination stimulating strigolactones (Daws *et al.* 2008). A relatively low fire response was also reported for species from the Mediterranean Basin (Crosti *et al.* 2006). Furthermore, results are consistent with the few fire studies reported from abandoned fields in renosterveld. Here, the total species number did not increase after burn but species composition changed with higher proportion of geophytes (Memiaghe 2008). Minor fire effects on species number were also observed compared to other restoration treatments (Midoko-Iponga 2004, Musil *et al.* 2005). There are different reasons that could explain the relatively low fire response for renosterveld. First of all, renosterveld species could be more affected by heat than smoke. Such independent fire response are reported from other Mediterranean-type ecosystems (Enright *et al.* 1997). Secondly, renosterveld species may be indeed more prone to other disturbances than to fire. This is supported by studies that questioned the universal germination enhancing effect of fire (Pierce *et al.* 1995) and the mentioned fire restoration studies (Midoko-Iponga 2004, Musil *et al.* 2005, Memiaghe 2008). Renosterveld has more fertile soil and palatable vegetation than fynbos and once supported large herds of game (Krug and Krug 2007). Therefore, it could have lead to disturbance adaptation rather than to pronounced fire or smoke response. Thirdly, the low germination magnitude might be due to suboptimal smoke primer concentration. While low smoke concentrations can enhance germination, germination inhibitions are reported for high concentration (Enright *et al.* 1997, Pérez-Fernández and Rodríguez-Echeverría 2003). From the restoration and conservation point of view results would support strategies that involve fire for renosterveld management. Fire seems to be a valuable tool to enhance species diversity and abundances. However, caution is needed in renosterveld habitats with high alien species presence. This is especially true for degraded sites, such as abandoned fields. Removing of alien grasses can be followed by appearance of more noxious species from the soil seed bank, such as *Echium plantagineum* (*sensu* Mau-Crimmins 2007). This dilemma can only be avoided by conducting vegetation and soil seed bank surveys prior to fire management interventions.

## 4 Does fire promote rapid germination adaptation in *Echium plantagineum* (Patterson's Curse)?

### Abstract

*Echium plantagineum*, native to the Mediterranean Basin, is an invasive annual herb in other Mediterranean-type ecosystems, such as South Africa and Australia. Local adaptations occur within the species, such as higher seedling establishment or high variation of traits and phenotypic plasticity in non-European populations. The study examined if these adaptations also apply for the germination response. Specifically, the influence of smoke-primer as a fire surrogate on germination percentage and rate on *E. plantagineum* seeds from France, South Africa and Australia was tested. A different germination response to smoke-primer treatment depending on seed origin and growth habitat was evident. The highest germination levels were found in South African populations from highly disturbed habitats along roads. This is interpreted as species response to higher frequencies of human-caused fires along roads and urbanized areas. Results underline the ability of invasive species to adapt their germination behavior in altered disturbance regimes, which should be considered for restoration and conservation management.

## Introduction

Invasive species negatively affect indigenous flora and biodiversity (Mooney and Cleland 2001, Henderson *et al.* 2006). This is especially true for Mediterranean-type ecosystems, where invasive plants have threatened regions of high species diversity and endemism. *Echium plantagineum* L. (Patterson's Curse), native for the European Mediterranean region, is known for its toxic compounds (Stegelmeier *et al.* 2009) and is one of the major non-woody invasive species in south-western Australia and South Africa (Nel *et al.* 2004). The species is of high research interest because of its immense invasibility and defying of control (Piggin 1976, 1978). Mediterranean-type ecosystems are fire-prone (Cowling *et al.* 1996) and *E. plantagineum* has plant-traits evolved with this disturbance regime.

Originating from the Mediterranean region of southern Europe, *E. plantagineum* shows a positive germination response after smoke-primer addition and fire application (Stevens *et al.* 2007). Interestingly, local adaptations occur within the species, for example seedling establishment is up to five times higher in south-eastern Australian populations compared to European populations from southern Portugal (Grigulis *et al.* 2001). Furthermore, Sharma and Esler (2008) showed the high variation of traits and phenotypic plasticity of the species in different habitats of South Africa. Specifically, they measured significant differences in plant and seed morphology between natural areas and disturbed sites along roads and concluded that the plastic response to different habitat types contributes to the invasiveness of *E. plantagineum*.

It was hypothesized that local adaptations of South African seeds from natural habitats and disturbed roadsides (Sharma & Esler, 2008) would also occur within the germination response. Additionally, seed material from the Mediterranean Basin and Australia was collected and compared with South African seeds. *Echium plantagineum* is adapted to Mediterranean-type climate (Piggin 1976) and since all three ecoregions are fire-influenced a smoke-primer treatment (fire surrogate) prior to germination applied. It was hypothesized that such treatment will enhance germination percentage and rate.

## Materials and Methods

### *Study species*

*Echium plantagineum* L. (Boraginaceae) is an erect annual (occasionally biennial), commonly 30–60 cm in height (maximum height=1.5m), that reproduces by seed. For detailed information see (Sharma and Esler 2008). It is commonly known as Patterson's Curse and is

native to southern Europe (Grigulis *et al.* 2001). Although generally a spring-flowering annual, *E. plantagineum* is highly adaptable and given suitable rainfall some plants germinate out-of-season and persist for longer than one year. It is a very prolific seed producer; heavy infestations can yield up to 10,000 seeds per square meter (Piggin 1978). It can germinate under a wide variety of temperature conditions, tolerates dry periods well, and responds vigorously to fertilizer. *Echium plantagineum* is introduced into Australia and southern Africa and is classified as a major invader that is common and widespread (Grigulis *et al.* 2001, Nel *et al.* 2004).

#### *Data collection and study-area*

*Echium plantagineum* were sampled from three Mediterranean-type ecosystems (Fig. 11) to observe the possible adaptive germination response of this species. In October and November 2008 mature seeds in a similar developmental stage were collected and stored in paper bags at dark and room temperature. One population from natural vegetation was sampled in France and one in Australia. In South Africa it was possible to sample and pool three populations from natural vegetation and along tarred roads. From every location approximate 20 plants were randomly sampled along a 100 m transect and 200 seeds were randomly chosen to conduct the following germination experiments.



**Fig. 11. Map of sampling date and location.**

Full circles show sampling locations. France: 5.10.2008 (43°00'08" N, 6°12'38" E), Australia: 6.11.2008 (31°45'19" S, 116°39'03" E), and South Africa: 8.11.2008 (natural areas 33°52'09" S, 18°35'84" E), (road sides 33°52'51" S, 18°45'41" E).

### *Germination experiment and data analyses*

For the germination experiments, five replicates (each replicate representing 20 seeds) of two treatments (with and without smoke-primer *Kirstenbosch Instant Smoke Plus*) were transferred to moist paper filters in Petri-dishes and put into germination chambers. A temperature regime of 22 °C during day (14 h) and 14 °C in the night (10 h) was applied. The effect of smoke primer application on germination rates within and between populations was analyzed by Mann-Whitney U-test and ANOVA. Temporal germination response or germination speed ( $gs$ ) within the first week was calculated as  $gs = (y_2 - y_1) / (x_2 - x_1)$ , which is the ratio between two points  $(x_1, y_1)$  and  $(x_2, y_2)$  on a line. All statistical analyses were performed using SPSS (SPSS Inc., Chicago, USA, v.15).

## **Results**

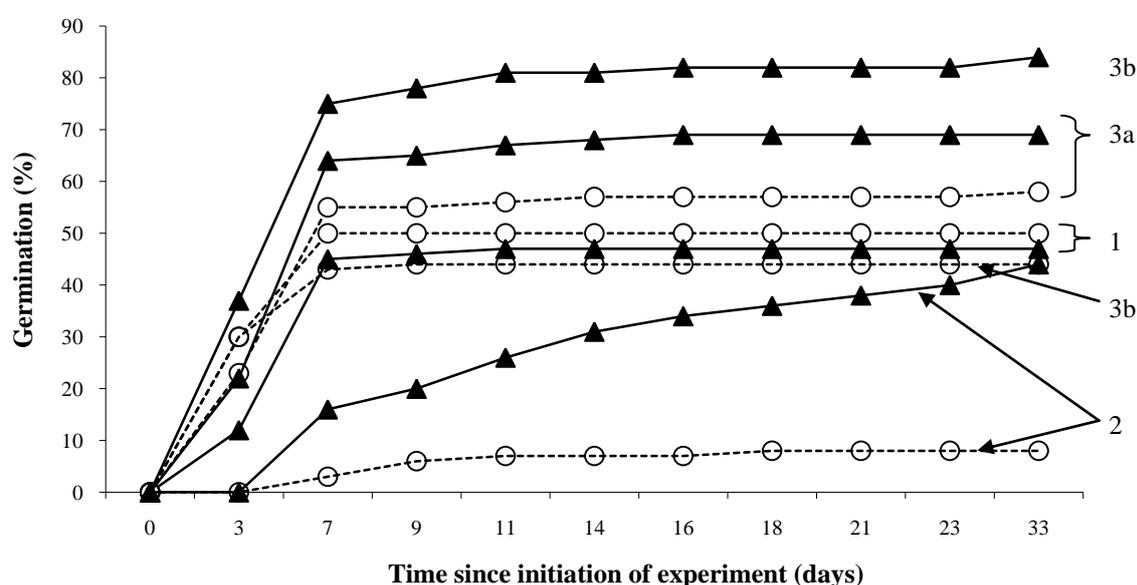
When applying smoke primer to *E. plantagineum* (Table 6), germination percentage of seeds from France (~50 %) and natural habitats in South Africa (~60 %) were not significantly different compared to non-smoke treatments. However, significantly higher germination percentage occurred in seed material from South African populations collected along roads (difference 40 %) and in material from Australia (difference 36 %). Highest germination percentage occurred in seed material from road habitats in South Africa, whereas lowest germination percentage occurred in seed material from Australia. Highest germination percentage with non-smoke treatment occurred in seed material from natural habitats in South Africa, whereas lowest germination percentage occurred in seed material from Australia. Within the non-smoke application no significant differences in germination percentage (~50 %) were recorded, except for Australia. Within the smoke-primer treatment significant differences occurred between South African populations collected along roads, South African natural habitats, France and Australia.

The germination rate were similar in non-smoke treatments except for Australian seeds that showed a significant lower germination rate compared to seeds from other origins (Fig. 12, Table 6). The germination rate within the smoke-primer treatment showed significant differences between seeds from all locations. Highest germination rate occurred in seeds from South African road side populations, while lowest rate was visible in Australian seeds.

**Table 6. Germination data of *E. plantagineum* from different Mediterranean habitats.**

Statistics show mean±S.E. Mann-Whitney *U*-test show sites comparison (\*\*= $p<0.01$ ). ANOVA show comparison within non-smoke and smoke treatment (\*\*\*= $p<0.001$ ; not significant = n.s.). Populations are either indigenous (I) or alien (A) and occurred in natural habitats (N) or along tar roads (R).

| Origin             | Germination percentage (%) |                       |                    | Germination rate       |                         |
|--------------------|----------------------------|-----------------------|--------------------|------------------------|-------------------------|
|                    | Non-smoke                  | Smoke-primer          | M-W <i>U</i> -test | Non-smoke              | Smoke-primer            |
| France (I, N)      | 50 ± 5.0 <sup>a</sup>      | 47 ± 4.6 <sup>a</sup> | 10.5 n.s.          | 7.1 ± 0.7 <sup>a</sup> | 6.5 ± 0.6 <sup>a</sup>  |
| South Africa (A,N) | 58 ± 5.8 <sup>a</sup>      | 69 ± 4.0 <sup>b</sup> | 6.0 n.s.           | 7.8 ± 0.6 <sup>a</sup> | 9.2 ± 0.6 <sup>b</sup>  |
| South Africa (A,R) | 44 ± 5.1 <sup>a</sup>      | 84 ± 1.9 <sup>c</sup> | 0.0 **             | 6.2 ± 0.7 <sup>a</sup> | 11.1 ± 0.2 <sup>c</sup> |
| Australia (A,N)    | 8 ± 2.0 <sup>b</sup>       | 44 ± 5.1 <sup>a</sup> | 0.0 **             | 0.9 ± 0.3 <sup>b</sup> | 2.8 ± 0.5 <sup>d</sup>  |
| ANOVA (df=3)       | $F=21.9$ ***               | $F=21.5$ ***          |                    | $F=26.7$ ***           | $F=53.3$ ***            |

**Fig. 12. Germination percentage of *E. plantagineum* from different Mediterranean habitats**

Smoke-primer application (filled triangles, full line) and non-smoke treatment (circles, dotted line). Number shows origin of seeds: 1-France, 2-Australia, 3a- South Africa (natural populations), 3b- South Africa (along roads).

## Discussion

The study revealed significant differences in the germination response between and within Mediterranean regions. Native *E. plantagineum* collected in France had a medium germination rate and did not respond to smoke primer treatment. This is interpreted as response to a relatively low fire frequency of 30-50 years in this region compared to 10-15 year fire intervals in South African fynbos (van Wilgen *et al.* 1992, Cowling *et al.* 1996). On the other hand, a significant increase in germination after smoke primer application was visible in South African seeds from road sides and compared to seeds collected from natural habitats. This interpreted as local adaptation to higher fire frequencies, which are due to human-induced ignition near urban areas and roads (Kalabokidis *et al.* 2002, Syphard *et al.*

2007, Archibald *et al.* 2009) or to occasional fire management along the tar roads (K. Esler, pers. comm.). Interestingly, invasive seeds from Australia responded greatly to smoke-primer, but had a low germination rate without smoke-primer application. This could indicate that *E. plantagineum* is already adapted to smoke-only germination in Australia. Results correspond with significant demographic and phenotypic differences between regions (Grigulis *et al.* 2001), between habitats within a region (Sharma and Esler 2008) and the positive fire influence on germination (Stevens *et al.* 2007). The temporal germination response was similar between regions and treatments with rapid germination occurring within a week. However, seeds collected in Australia showed a less steep germination response, which could indicate non-ideal temperature regimes during the experiments. The findings strengthen the findings of former studies on high plasticity of invasive species. From a management perspective this study shows that fire treatment in conservation and restoration is a critical tool and should be avoided, if infestation with *E. plantagineum* is present.

## 5 Pioneers, perches and pine clearing - promising restoration methods of degraded renosterveld habitats?

### Abstract

Areas of abandoned agricultural fields and pine stands are globally increasing and also common features in the Cape Lowlands of South Africa. Previous restoration attempts on degraded Western Cape Centre renosterveld have been of little success and therefore novel approaches are needed for this area. The study reports on three restoration experiments, designed to re-introduce key functional plant types back into this critically endangered habitat. The first experiment in this study concentrated on a common pioneer species in renosterveld vegetation, *Otholobium hirtum*. Although *in-vitro* experiments showed a significantly elevated germination response after scarification and smoke primer treatment, *in-vivo* experiments have failed to deliver establishment in abandoned field. The second restoration experiment focussed on bush clumps, a sub-type of renosterveld vegetation that is characterized by broad-leaved shrubs with fleshy bird-dispersed diaspores. The effect of artificial bird perching structures and their potential to enhance diaspore dispersal by frugivorous birds in degraded renosterveld plant communities was tested. Results showed a significant increase of seed dispersal at perched sites. However, *in-vivo* seed germination and establishment in abandoned fields was not recorded. In a third experiment, clearing of a pine plantation was surveyed. Data showed that recovery of indigenous flora was high because the soil seed bank was not depleted and soil parameters were similar to an adjacent pristine site. The experiments revealed that restoration using natural vectors face immense problems, despite their promising potential. Before launching large-scale restoration programs in abandoned fields, preliminary studies are strongly recommended. Nevertheless, cleared pine plantations on former renosterveld have a high restoration potential and should be prioritized for restoration.

## Introduction

Abandoned fields and pine stands are increasing features of anthropogenic altered ecosystems (Richardson *et al.* 1994, Young 2000, Cramer *et al.* 2007). There are also potential restoration sites with restoration success strongly depending on abiotic and biotic factors (Saunders *et al.* 1991), as well as on temporal and spatial dispersal of diaspores (Poschlod *et al.* 1998). In general, restoration should quickly produce particular target conditions and maintain them for long time frames (D'Antonio and Meyerson 2002). Across vegetation zones, several attempts have been made to re-introduce local species into abandoned field by various methods (e.g. mowing, burning, transplanting and perching) and such efforts are often accompanied with clearing of alien species using cutting, burning and herbicide application (van Andel and Aronson 2006).

The study reports on methods to re-introduce indigenous species into degraded habitats of critically endangered West Coast Centre renosterveld; a Mediterranean-type shrubland in the Fynbos biome (*sensu* Di Castri and Mooney 1973, Specht and Moll 1983). The main component of renosterveld is a shrub matrix characterized by asteraceous wind-dispersed species and intermingled by termitarias, locally called “heuweltjies” and habitat of bird-dispersed shrubs or trees species (Rebelo *et al.* 2006). Renosterveld has been heavily transformed by agriculture (Cowling and Pierce 1999, Heijnis *et al.* 1999, Kemper *et al.* 1999) and abandoned fields are a common feature of the region (Krug and Krug 2007, Newton 2008) showing slow succession and recovery of indigenous plant species due to soil degradation (Milton 2004) and alien grass competition (Midoko-Iponga 2004, Midoko-Iponga *et al.* 2005). To a lesser extent, pine plantations are found in former renosterveld areas and able to invade natural vegetation, thereby reducing species diversity and negatively impacting on plant communities, soil and water resources (Macdonald and Richardson 1986, Richardson *et al.* 1994, Le Maitre *et al.* 2002).

However, a “memory” of pre-degraded vegetation could be still viable in form of a soil seed bank (Bakker *et al.* 1996), which represents a potential diaspore source for restoration attempts (Willems and Bik 1998; see also chapter two, this thesis). Generally, succession depends on abiotic factors (e.g. rainfall and temperature), past land use type and disturbance intensity (e.g. fire frequency and soil conditions) and the surrounding landscape matrix (Holl *et al.* 2000). New plant establishment results from current availability of seed rain, existing seedlings and seed bank, as well as resprouting plants. Although plant species are able to persist in small remnants within a matrix dominated by agriculture (Cowling and Bond 1991, Kemper *et al.* 1999), it is necessary to buffer and enlarge those to meet conservation goals, to

protect them from further habitat loss and transformation, as well as to prepare for climate change impact.

There is an urgent need to develop new management tools in order to respond to highly competitive alien grass infestation of abandoned fields (Krug *et al.* 2004a, Milton 2004, Sharma *et al.* 2010). Until now, research has focused on methods to reduce alien grass abundance (e.g. Musil *et al.* 2005, Ruwanza 2008). In order to restore a particular habitat it is necessary to re-introduce key elements that provide structural features of the original habitat (Miller and Hobbs 2007). Here, two methods novel to the Cape region are presented that could introduce a much needed structural component into degraded habitats. Following these strategies, establishment of shrub matrix into abandoned fields could be enhanced, thereby combating alien grass infestation.

A particular pioneer species, *Otholobium hirtum* (Fabaceae), has the ability to outcompete dominant alien grasses and create microhabitats for indigenous plant species in abandoned fields (pers. observ.). In the first restoration experiment presented, seeds of *O. hirtum* were collected and subjected to different germination treatments, in order to deepen knowledge of autecology of this pioneer species (Levyns 1935, Rebelo 1995) and to find optimal conditions for germination experiments. Germination and establishment were tested under field conditions using combined treatments (grass elimination, herbivore exclosure, and seed scarification).

Birds are important dispersal vectors and attracted to numerous perch structures where defecation and regurgitation takes place (Jordano and Schupp 2000). The pattern of higher seed concentration beneath perches is characteristic for Mediterranean and European shrublands (Debussche *et al.* 1982, Izhaki *et al.* 1991, Debussche and Isenmann 1994, Herrera *et al.* 1994, Kollmann and Pirl 1995, Verdú and García-Fayos 1996). Modifications of the conventional seed shadow (Janzen 1971) can appear in such patchy habitat structure (Hoppes 1988, Debussche and Lepart 1992, Debussche and Isenmann 1994, Kollmann and Pirl 1995, Aguiar and Sala 1997) with seed vectors inducing nucleation processes (Willson and Crome 1989, McClanahan and Wolfe 1993, Verdú and García-Fayos 1996, Julliot 1997). While many studies have looked at perch and nucleation effects (Verdú and García-Fayos 1996) only few have dealt with bird mediated restoration (Handel 1997) and restoration potential of artificial perching structures in Mediterranean (Vallejo *et al.* 2005) and tropical ecosystems (Holl 1998, Holl *et al.* 2000). Pausas *et al.* (2006) highlighted the need to mimic natural processes (both structural and functional) for active management strategies as well as to enhance landscape recovery via artificial perching structures. However, it is known that

dispersal of fleshy fruits is a limiting factor in recolonization of abandoned fields (Kollmann and Pirl 1995, Grunicke 1996, Shea 2007). In South Africa, Knight and Siegfried (1983, 1988) were the first to study the perch effect in cleared mountain fynbos vegetation, followed by studies in coastal fynbos (Cowling *et al.* 1997a). They have found that perches, such as shrubs and telegraph lines can enhance densities of bird dispersed species. Unfortunately, only few data on post-dispersal establishment exist and no such data are available for renosterveld. The second restoration experiment tested, if artificial bird perches enhance seed dispersal into abandoned fields on former West Coast Centre renosterveld, thereby mimicking nucleation processes (Connell and Slatyer 1977, Verdú and García-Fayos 1996). Since bird dispersal alone does not imply successful species establishment (Holl *et al.* 2000), it was tested if reduction of grass by weeding has an effect of germination success.

In a third experiment, pine clearing in the Tygerberg Nature Reserve was surveyed and its restoration potential (i.e. re-appearance of non-alien flora) evaluated. In general, alien-clearing results in good recovery of indigenous species, such as fynbos (Fourie 2008) and natural forests (Geldenhuys 1997). It is suggested that alien removal and regeneration with indigenous flora work well in renosterveld (De Villiers *et al.* 2005). However, no such study is known to us, except for positive observation after restoration at Papegaaiberg near Stellenbosch (C. Boucher pers. comm.). All three experiments could give insights into future renosterveld restoration methods and priorities.

## Material and Methods

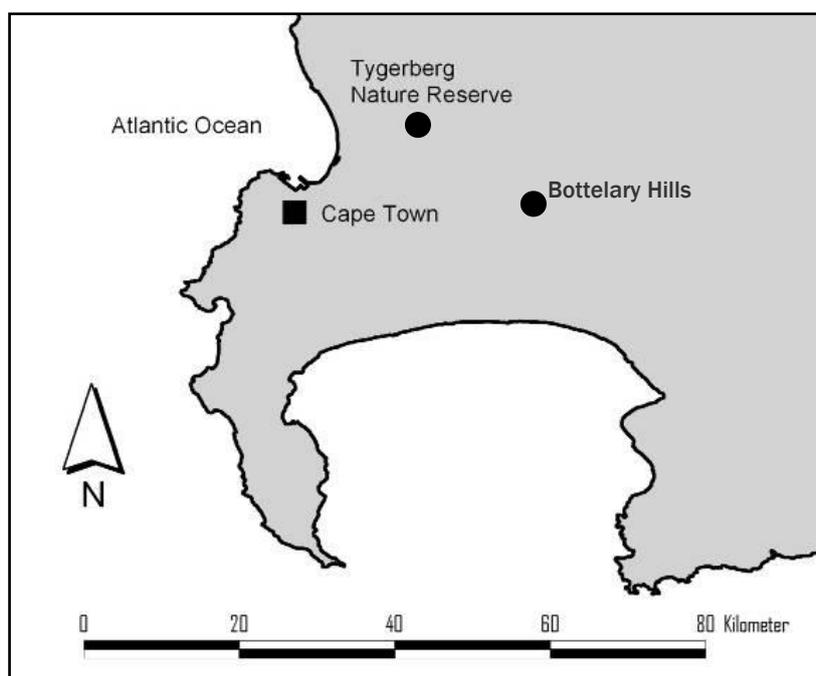
### *Study area and sites*

The study was performed at the Tygerberg Nature Reserve (Tygerberg Hills, S -33.87; E 18.59) and Mooiplaas Wine Estate (Bottelary Hills, S -33.93; E 18.74) on the Cape Lowlands of South Africa (Fig. 13). Both sites are typical representatives of remaining Western Cape Centre renosterveld and adjacent to abandoned fields or pine stand. The area is characterized by Mediterranean-type climate and nutrient-rich and alkaline soils with high clay content.

### *Pioneer experiment*

The first experiment was performed at Tygerberg Nature Reserve. Cover values of *Otholobium hirtum* and Poaceae spp. were measured along ten 6 m-transects from the core of an *O. hirtum* patch towards an abandoned agricultural field. Seed collection for germination experiments of *O. hirtum* took place in April 2007. Members of the Fabaceae family require a

pre-germination heat shock or other stratification in order to overcome physical dormancy caused by impermeable seed coat (Baskin and Baskin 1998, Hanley et al. 2001, Van Assche et al. 2003). Pre-germination treatments included scarification using sandpaper and heat shock (10 minutes at 60 °C) via a modified drying chamber (Bylebyl 2007). Sixteen in-vitro germination experiments with eight replicates each containing 15 seeds were carried out in climate chambers (Table 7). *In-vivo* germination experiments took place at Tygerberg hills. Ten experimental split plots were installed in August 2009 with sowing of partly scarified seeds (1 kg), manual weeding (mimicking bioturbation) and enclosure (mesh size 1 cm, test for herbivore influence).



**Fig. 13. Map of the Cape lowlands, South Africa.**  
Dark dots indicate study sites at Tygerberg and Bottelary Hills.

**Table 7. Treatment regimes and codes for *Otholobium hirtum*.**

LD=Light/dark treatment, D=Dark treatment. Treatment codes are shown bold.

| Treatment parameter |           |    | Ambient temperature<br>with scarification |            | 60°C heat shock<br>with scarification |            |
|---------------------|-----------|----|---|------------|---------------------------------------|------------|
|                     |           |    | yes                                       | no         | yes                                   | no         |
| 20 °C/12 °C         | 12 h/12 h | LD | <b>A 1</b>                                | <b>A 2</b> | <b>B 1</b>                            | <b>B 2</b> |
| 20 °C/12 °C         | 12 h/12 h | D  | <b>A 3</b>                                | <b>A 4</b> | <b>B 3</b>                            | <b>B 4</b> |
| 20 °C/20 °C         | 12 h/12 h | LD | <b>A 5</b>                                | <b>A 6</b> | <b>B 5</b>                            | <b>B 6</b> |
| 20 °C/20 °C         | 12 h/12 h | D  | <b>A 7</b>                                | <b>A 8</b> | <b>B 7</b>                            | <b>B 8</b> |

*Perch experiment*

The second experiment was performed at Tygerberg and Bottelary Hills, each comprising 10 perches and seed traps erected in abandoned agricultural fields. Modified net seed traps (1.2 m x 1.2 m) at the soil surface and situated below each perch (Fig. 14) were used to measure the input of bird faeces and bird-dispersed seeds, as well as a control in the open field (*sensu* Bullock *et al.* 2006). Nets had a mesh size of 2 mm to allow drainage but prevention of seed loss and were supported on 20 cm stone piles. Cut branches of *Eucalyptus* sp. were planted into the soil as artificial perch. Mean height of the branches was 212 cm and a mean diameter was 128 cm. Prior to the next fruiting season traps were removed. Two quarter of the area below each perch were cleared from alien grasses in order to study seedling establishment in vegetation and competition-free sites.



**Fig. 14. Artificial bird perching structure with netted seed trap.**  
Design adapted from Smith (1975) and McDonnell & Stiles (1983).

*Pine clearing experiment*

The third experiment was performed at Kanonberg, a section within the Tygerberg Nature Reserve. Felling and burning of a pine plantation took place in 2008 (Fig. 15). Vegetation surveys and soil analyses were carried out at the cleared site and adjacent pine plantation and pristine renosterveld vegetation.



**Fig. 15.** Restoration site immediately after pine clearing (A) and one year later (B).

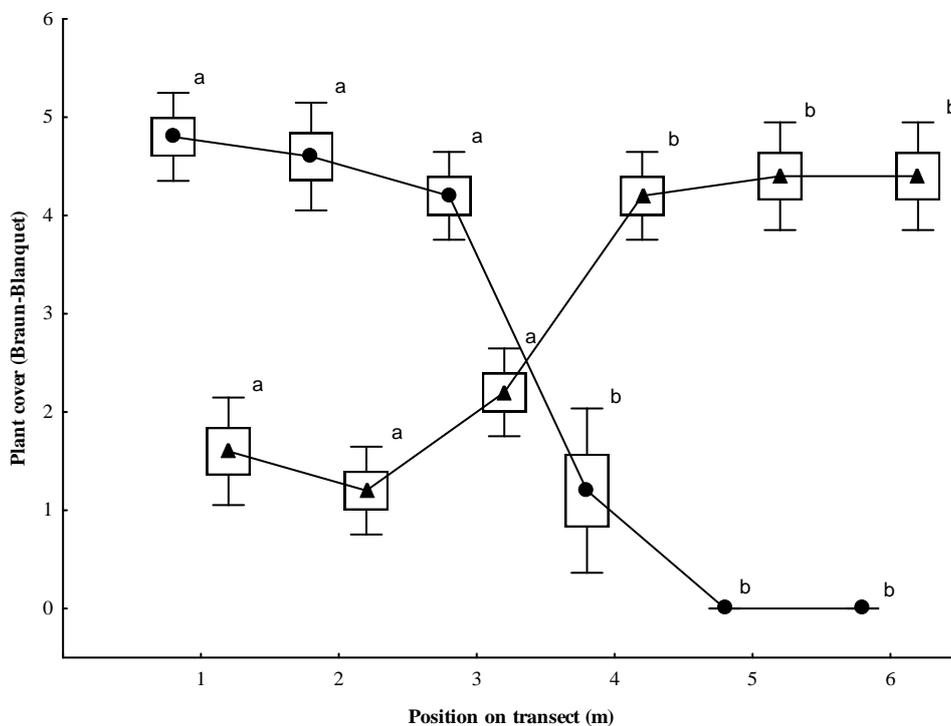
### *Numerical analyses*

Non-parametric data were analysed for statistically significant differences using the Kruskal-Wallis  $H$  test and Mann-Whitney  $U$  test including Bonferroni correction. All statistical tests were computed with SPSS (SPSS Inc., Chicago USA, v17) and graphs were generated with Microsoft Excel 2007. DCA ordination was generated with PC-Ord 4.2.

## Results

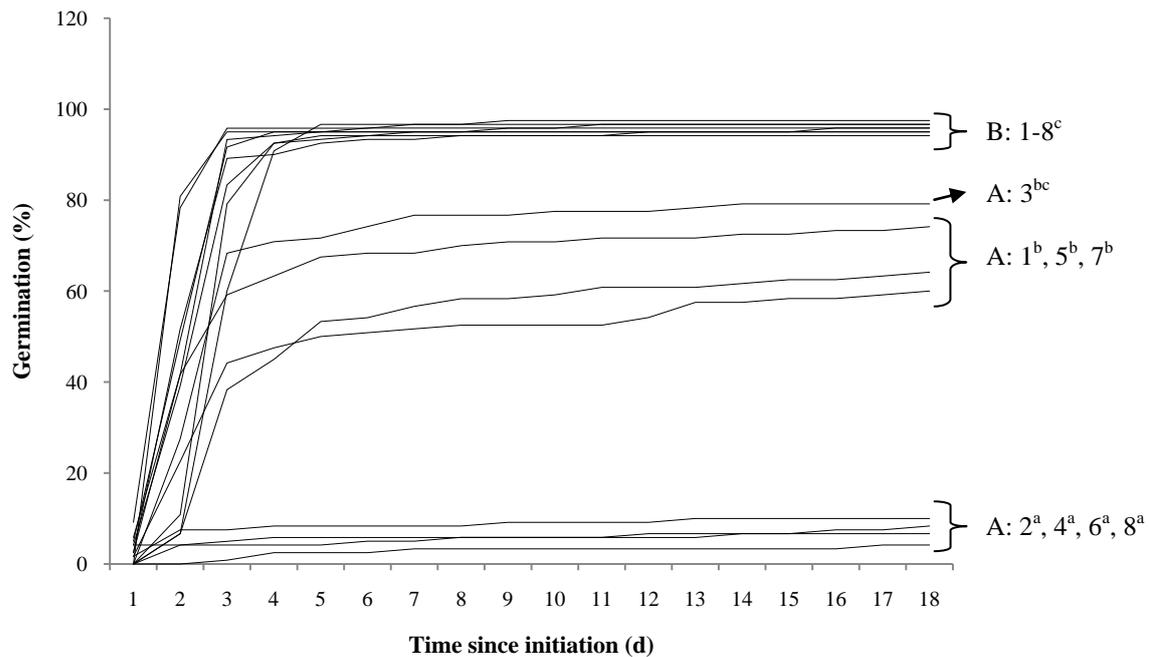
### *Pioneer experiment*

*O. hirtum* is able to significantly reduce grass cover (Fig. 16). Germination experiments (Fig. 17) showed a significant response of scarification that enhanced germination rates from 10 % (A2, 4, 6, and 8) up to 80 % (A1, 3, 5, and 7). Heat-shock treatment significantly increased germination rates to near 100 % (B1-8). Only the A3 treatment (scarification, 20 °C/12 °C, D) did not show a significant difference compared to the heat-shock treatment. Unfortunately, *in-vivo* germination and establishment experiment failed to deliver results since no seedlings established.



**Fig. 16. Plant cover values of *Otholobium hirtum* and Poaceae spp.**

*Otholobium hirtum* (circle) and Poaceae spp. (triangle). Transect from *O. hirtum* patch core (1m) towards abandoned agricultural field (6m). Box-Whisker plot show mean, S.E. and S.D. Kruskal-Wallis  $H$  test ( $H=26$  shrub;  $H=25$  grass;  $p < 0.001^{***}$ ). In between group comparison with sequential Mann-Whitney  $U$  test including Bonferroni-correction. Different letters indicate statistically significant differences between plant cover values of *O. hirtum* and Poaceae.



**Fig. 17. Germination treatments of *Otholobium hirtum*.**

With ambient temperature (A) and 60°C heat-shock (B). For treatments regimes and codes see Table 7. Results of Kruskal-Wallis  $H$  test ( $H=106.0$ ;  $N=128$ ;  $p<0.001$ ). Significant results of subsequent pair-wise Mann-Whitney  $U$  test including Bonferroni correction are shown as different letters ( $p<0.001$ ). Fifteen seeds per replicates and eight replicates per treatment.

#### *Perch experiment*

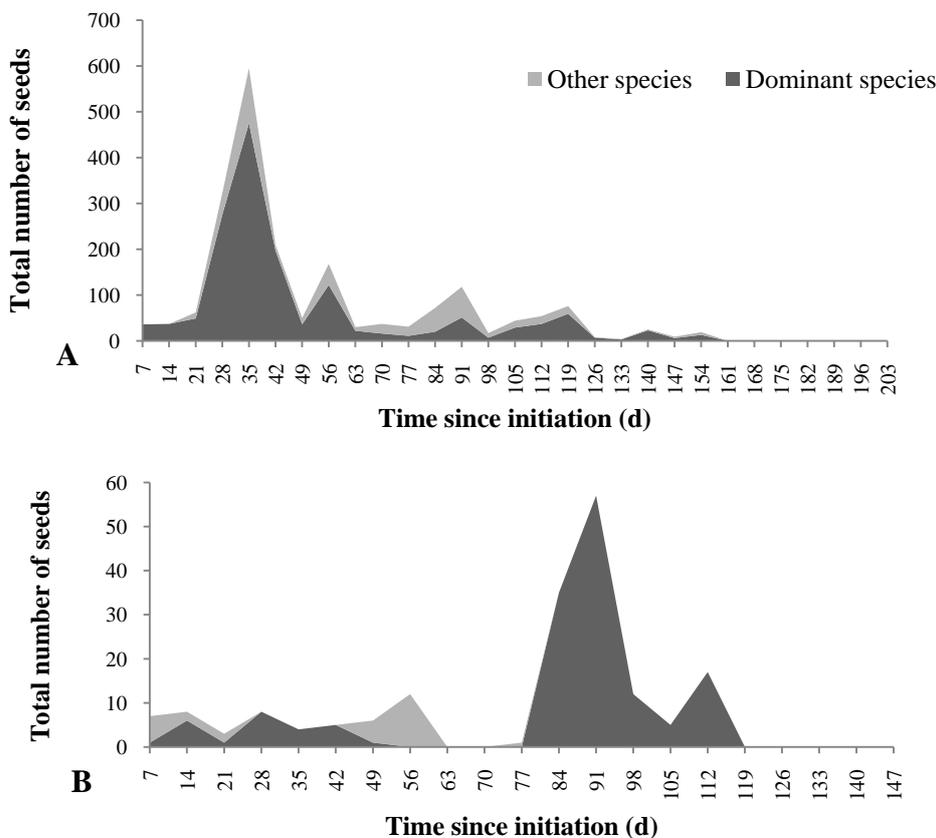
At Tygerberg Nature Reserve, 12 hours of bird observations were carried out between 15.01.-14.04.2008. In this period, 16 bird species were recorded in the area (*Ardea cinerea*, *Buteo rufofuscus*, *Colius colius*, *Colius striatus*, *Corvus albus*, *Cuculus solitarius*, *Elanus caeruleus*, *Falco tinnunculus*, *Guttera purcherani*, *Hirundu cucullata*, *Lanius collaris*, *Laniarius ferrugineus*, *Nectarinia chalybea*, *Numida meleagris*, *Prinia hypoxantha*, unidentified sp.), 9 of them having a partly frugivorous feeding behaviour. *Lanius collaris*, a partially frugivorous species, was the only bird species visiting the artificial perches (21 times for a total duration of 45 minutes). Seeds of 11 plant species (179 seeds in total) were found in netted seed traps under perches during the experimental period of 9 month (Table 8, Fig.18). At Mooiplaas Wine Estate, 22 hours of bird observations were carried out between 07.10.-19.03.2009. In this time period, 11 bird species were recorded (*Prinia maculosa*, *Euplectes capensis*, *Serinus canicollis*, *Serinus flaviventris*, *Sigelus silens*, *Cisticola textrix*, *Apalis thoracica*, *Pycnonotus capensis*, *Cercotrichas coryphaeus*, *Lanius collaris*, *Cisticola fulvicapilla*), 5 of them having a partly frugivorous feeding behaviour. Those bird species visited artificial perches 109 times for a total duration of 91 minutes. Seeds of 12 plant species (2024 seeds in total) were found in netted seed traps under perches during the experimental

period 6 month period (Table 8, Fig.18). At both sites the mean number of seeds and faeces was significantly higher under the perches compared to the control (Table 8). Between sites, Mooiplaas had significant higher mean number of seeds compared to Tygerberg (Table 8). The subsequent removal of seed traps and successive seed deposition by birds the next fruiting season were not followed by germination of shrubs in vegetation and vegetation-free sites below the perch.

**Table 8. Dispersal of diaspores at Mooiplaas and Tygerberg.**

Totals or means and standard error of means are given. Mann-Whitney *U* test, \*\*\*\*  $p < 0.001$ ,  $N = 10$ . Significant higher values of site comparison are bold indicated bold.

|                   | Mooiplaas (A)           |           | Tygerberg (B)     |           |
|-------------------|-------------------------|-----------|-------------------|-----------|
|                   | Perch vs. Control       |           | Perch vs. Control |           |
| Total no. species | 12                      |           | 10                |           |
| Total no. faeces  | 4316                    | 15        | 829               | 24        |
| Total no. seeds   | 2024                    | 0         | 179               | 0         |
| Mean no. faeces   | 431,6 ± <b>86,8****</b> | 1,4 ± 0,3 | 82,9 ± 16,5****   | 2,4 ± 0,5 |
| Mean no. seeds    | 202,4 ± <b>72,4****</b> | 0,0 ± 0,0 | 17,9 ± 6,2****    | 0,0 ± 0,0 |



**Fig. 18. Number of dispersed seeds at Mooiplaas and Tygerberg.**

Mooiplaas (A) and Tygerberg (B). Dominant species (seed number > 200) Mooiplaas: *Olea europaea* ssp. *africana*, *Osteospermum moniliferum*, *Rhus* sp.1. Dominant species (seed number > 20) Tygerberg: *Euclea* sp. and *Rhus* sp. Other species for A: *Rhus* sp.2, *Acacia cyclops*, Indet (1-7). Other species for B: *Olea europaea* ssp. *africana*, *Osteospermum moniliferum*, *Medicago polymorpha*, *Rorippa palustris*, Indet (1-5). Note different scale and species.

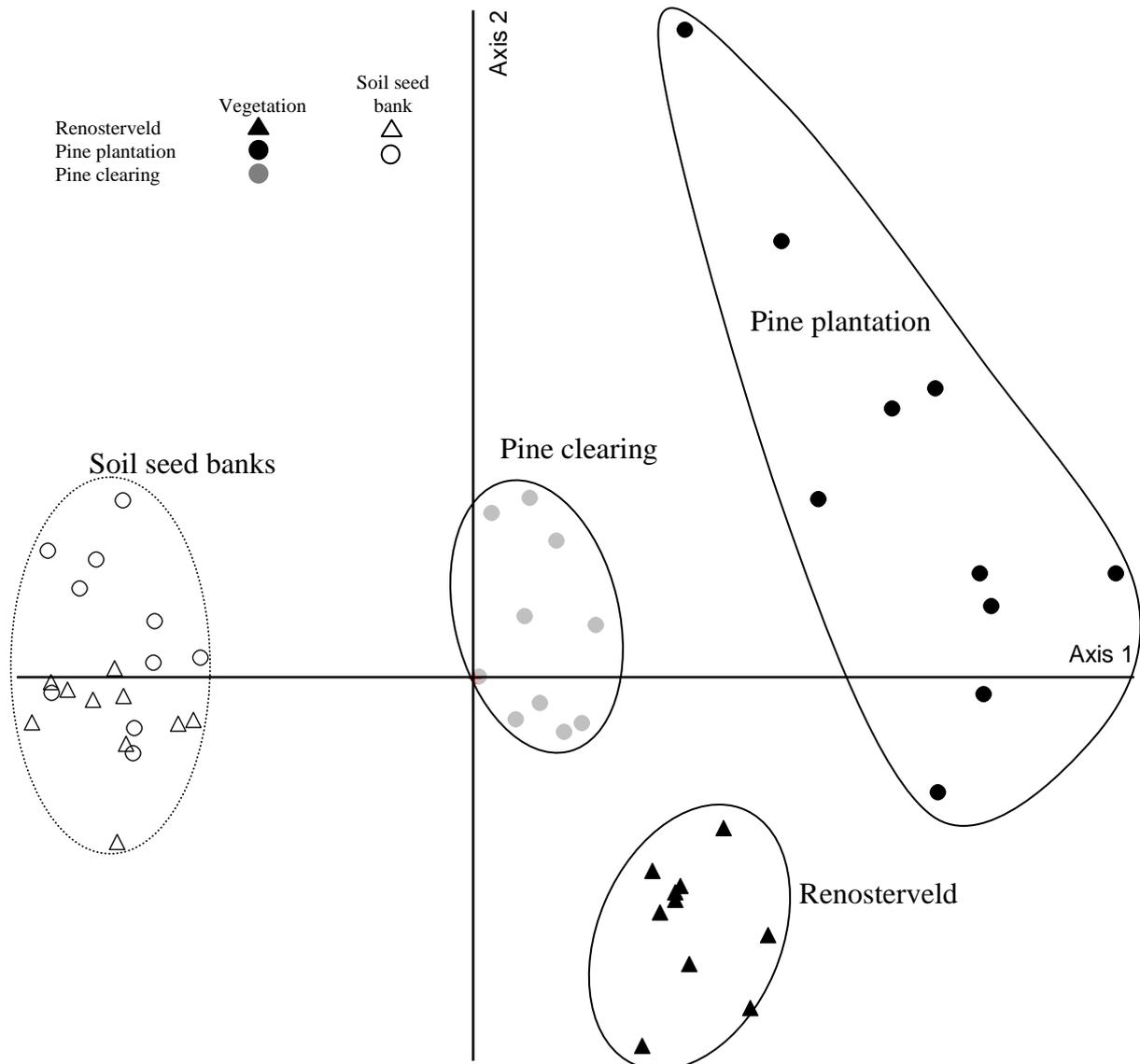
*Pine clearing experiment*

The soil analysis between adjacent management areas (e.g. pine plantation, cleared site, pristine renosterveld) revealed statistically significant changes in all soil parameters, except for carbon (Table 9). The majority of soil parameters did not show differences between cleared site and renosterveld. DCA-analysis showed four groupings that corresponded with different management areas and the soil seed bank (Fig. 19). Few individuals of alien and problematic *Pinus* sp. and *Acacia* sp. appeared after clearing. Species noted in the area but not appearing within plots were *Aristea africana* (Iridac.), *Zantedeschia aethiopica* (Arac.), *Gladiolus alatus* (Iridac.), *Gladiolus watsonius* (Iridac.), *Corycium orobachiodes* (Orchidac.), *Disperis villosa* (Orchidac.), *Chrysanthemoides monilifera* (Asterac.), *Salvia africana-lutea* (Lamiac.). Full species list of vegetation and soil seed bank is available in the Appendix.

**Table 9. Comparison of soil parameters and vegetation in pristine and degraded renosterveld.**

Means, standard errors of means, chi-square values of Kruskal-Wallis *H* Test and p-values (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001, n.s. – not significant, n.a. - not applicable) are given. Bold number show significantly different values from pine plantation (subsequent Mann-Withney *U* tests including Bonferroni correction). Cation exchange capacity with T-value: sum of exchangeable sodium, potassium, calcium, magnesium and hydrogen (CEC). Sampling size (N=10), except for clay, silt and sand (n=5).

|                    | Pine plantation            | Pine clearing                    | Renosterveld                      | Statistics |
|--------------------|----------------------------|----------------------------------|-----------------------------------|------------|
| pH                 | 4.88 ± 0.07 <sup>a</sup>   | <b>5.36 ± 0.12<sup>b</sup></b>   | <b>5.41 ± 0.07<sup>b</sup></b>    | 14.44 **   |
| P (mg/kg)          | 8.4 ± 0.40 <sup>a</sup>    | <b>5.6 ± 1.81<sup>b</sup></b>    | <b>5.5 ± 0.45<sup>b</sup></b>     | 9.98 **    |
| K (mg/kg)          | 294.8 ± 10.52 <sup>a</sup> | <b>237.0 ± 19.26<sup>b</sup></b> | <b>259.4 ± 14.28<sup>ab</sup></b> | 6.10 *     |
| N (%)              | 0.35 ± 0.03 <sup>a</sup>   | <b>0.16 ± 0.01<sup>b</sup></b>   | <b>0.19 ± 0.01<sup>b</sup></b>    | 18.93 ***  |
| C (%)              | 2.23 ± 0.31 <sup>a</sup>   | 1.97 ± 0.16 <sup>a</sup>         | 2.17 ± 0.05 <sup>a</sup>          | 1.30 n.s.  |
| C:N ratio          | 6.22 ± 0.42 <sup>a</sup>   | <b>12.21 ± 0.78<sup>b</sup></b>  | <b>11.4 ± 0.42<sup>b</sup></b>    | 17.51 ***  |
| CEC (cmol/kg)      | 15.37 ± 0.84 <sup>a</sup>  | <b>6.63 ± 0.41<sup>b</sup></b>   | <b>8.49 ± 0.48<sup>b</sup></b>    | 17.33 ***  |
| Clay (%)           | 2.12 ± 0.28 <sup>a</sup>   | 4.28 ± .89 <sup>a</sup>          | 1.00 ± 0.00 <sup>b</sup>          | 10.35 **   |
| Silt (%)           | 4.80 ± 1.20 <sup>a</sup>   | <b>10.72 ± 1.09<sup>b</sup></b>  | <b>11.40 ± 1.40<sup>b</sup></b>   | 8.16 *     |
| Sand (%)           | 92.80 ± 1.20 <sup>a</sup>  | 84.40 ± 1.91 <sup>b</sup>        | 87.60 ± 1.40 <sup>a</sup>         | 7.97 *     |
| Total no. spp.     | 59                         | 69                               | 92                                | n.a.       |
| Mean no. spp./plot | 13.8 ± 0.4 <sup>a</sup>    | <b>23.3 ± 0.3<sup>b</sup></b>    | <b>27.6 ± 0.6<sup>b</sup></b>     | 17.58 ***  |



**Fig. 19. DCA analysis of vegetation and soil seed bank (renosterveld, pine plantation, pine clearing).**

Power-transformed matrix of 173 taxa and 50 plots. Vegetation of pine plantation (black circles), pine clearing (grey circle), renosterveld (black triangles), soil seed bank of renosterveld (empty triangles) and pine plantation (empty circles). Increment (axis 1: 0.51, axis 2: 0.09, log: 0.44).

## Discussion

A high level of habitat degradation and competition with alien species diminish the suitability for re-establishment of species once present (Miller and Hobbs 2007). Therefore, abandoned fields and pine plantations persist in a degraded state and face severe ecological, philosophical and policy challenges (Cramer *et al.* 2008, Lindenmayer *et al.* 2008). A trial and error approach can provide practical lessons for restoration practice (Walker and del Moral 2008). Although field observations and germination experiments were promising, *in-vivo*

establishment of pioneer and bird-dispersed species in degraded renosterveld habitats have failed. As a consequence, both methods do not appear suitable as management and restoration option for abandoned fields. However, restoration of pine clearing using a cut and burn technique showed high recovery with indigenous flora and therefore should be set as restoration priority.

#### *Pioneer experiment*

A thermal shock modifies hard-coated seeds and overcomes physical dormancy (Baskin and Baskin 1989). This could be shown for Fabaceae seeds in many Mediterranean-type ecosystems (Hanley *et al.* 2001). Mechanical scarification has a similar influence in cracking the hard seed coat (Van Assche *et al.* 2003). Germination results are consistent with other studies and show the dependence of Fabaceae on stratification (e.g. pre-heating, scarification) in order to break physical dormancy (Bradstock and Auld 1995, Keeley and Bond 1997, Herranz *et al.* 1998). Despite the high *in-vitro* germination rates after scarification with sandpaper, *Otholobium hirtum* was not able to establish in field conditions. Main reason for establishment failure could have been herbivory of mice and mole rats, despite the installation of exclosures. Other factors include soil compaction (Verdú and García-Fayos 1996) and competition from grasses, which can suppress establishment of indigenous shrub species (Eliason and Allen 1997). Furthermore, *O. hirtum* also seem to be dependent on mycorrhiza for phosphorus uptake (Allsopp and Stock 1992, 1994) and vesicular-arbuscular mycorrhizal fungi could be reduced in abandoned fields. Large stands of *O. hirtum* appeared after fire in other localities (pers. observ.) and therefore fire (i.e. combined effects of smoke, heat and elimination of competitors) might be necessary for germination in field conditions. Although Aide and co-workers (1995) suggested a similar restoration strategy for pastures on former tropical rainforest, it cannot be recommended for abandoned fields in renosterveld.

#### *Perch experiment*

According to Philogene (1995) Tygerberg Hills sustain 171 bird species and a similar magnitude can be assumed for the Bottelary Hills. This rich ornithological fauna could act as dispersal vectors for fleshy diaspores of indigenous shrubs, thereby concentrating seeds beneath bird perches. Although seed rain was enhanced via bird perches, seed establishment failed. Generally, it is difficult to overcome establishment barriers such as seed predation, low germination ability and unfavourable germination conditions (Smith 1975, Kollmann 1994, Grunicke 1996, Holl *et al.* 2000, Herrera and García 2010). Results are coherent with findings from other regions where most studies reported a low seed establishment success (Table 10).

Suspected reason for experiment failure were soil compaction (Verdú and García-Fayos 1996) and competition with grasses, which suppress establishment of indigenous shrub species (Eliason and Allen 1997). However, establishment of dispersed seeds failed even within competition-free and tilled microsites. It seems that low germination rates of fruity seeds (R. Knight, pers. comm.; Traveset 1998) and seed predation by rodents are the main factor hindering seedling establishment (Hulme 1998). It is therefore questionable if further restoration efforts using birds as dispersal vectors (Wunderle 1997) should be directed to abandoned fields, especially if seed rain may contain alien species (Gosper *et al.* 2005).

**Table 10. Perch effect with enhanced seed rain in abandoned agricultural fields.**

<sup>#</sup>Enhanced germination success (+), no or low germination success (-), not available (n.a.).

| Ecosystem                          | Country      | Perching structure and site<br>(artificial-A, natural-N) | Germination success | Author   |
|------------------------------------|--------------|--|---------------------|--|
| Tropical<br>rainforest             | Costa Rica   | A (crossbar, branches),<br>abandoned fields              | -                   | (Holl 1998)  |
|                                    | Costa Rica   | N (windbreaks), pasture                                  | n.a.                | (Harvey 2000)  |
|                                    | Costa Rica   | A (n.a.)   | n.a.                | (Ferguson 1995)  |
|                                    | Colombia     | N (tree), abandoned fields                               | -                   | (Aide and Cavelier 1994)   |
|                                    | Honduras     | N (trees)  | -                   | (Zahawi and Augspurger 2008)   |
| Mediterranean<br>shrubland         | France       | N (trees), abandoned orchard                             | +                   | (Debussche <i>et al.</i> 1982)   |
|                                    | France       | N (trees), natural vegetation,<br>abandoned fields       | -                   | (Debussche and Isenmann 1994)  |
|                                    | Spain        | N (trees), abandoned fields                              | +                   | (Pausas <i>et al.</i> 2006)  |
|                                    | South Africa | A (telephone wire), N (trees)                            | -                   | (Knight and Siegfried 1983, Knight 1988)                               |
|                                    | South Africa | A (branches)   | -                   | This study   |
| Temperate<br>deciduous<br>woodland | USA          | A (crossbar, pile, branches),<br>abandoned fields        | n.a.                | (McDonnell and Stiles 1983)  |
|                                    | USA          | A (trees)  | n.a.                | (McDonnell 1986)   |
|                                    | USA          | A (trees), mining site                                   | -                   | (McClanahan and Wolfe 1993)  |
|                                    | USA          | A (trees), land fill                                     | +                   | (Robinson and Handel 1993)   |
|                                    | Germany      | N (shrubs), cleared vineyard,<br>abandoned land          | -                   | (Kollmann 1994, Kollmann and<br>Wilmanns 1994, Kollmann and Pirl 1995) |
|                                    | Germany      | A (bar)  | -                   | (Grunicke 1996)  |

*Pine clearing experiment*

Renosterveld is very susceptible to invasion of pine species and clearing is an appropriate tool to avoid further spread by eliminating the seed source and simultaneously creating new renosterveld habitats (Boucher 1984). Estimations by Kruger (1982) are the only existing data on the actual extent of pine plantation in renosterveld (23 km<sup>2</sup>). Nowadays, vast pine populations are present especially at Tygerberg and Bottelary Hills. In fynbos, “clearing and burning” of cut exotics are more negative for species richness than the “burn standing, cut, remove” approach, though managers often have few options (Britton and Jackelman 1995, Holmes *et al.* 2000, Cilliers *et al.* 2004). Pine clearing at Kanonberg with removal of the largest logs and burning of small fuel loads showed great recovery of soil parameters and indigenous flora. Clearing of pine plantations will immediately improve watershed protection and lower fire hazard (Le Maitre *et al.* 2002). This should encourage reserve managers to see pine plantations as a restoration option with high recovery potential for indigenous flora. However, monitoring and follow up clearing is required because establishment of alien woody species is still possible.

## Appendix

Species list of pine clearing experiment. Vegetation data shows mean Braun-Blanquet cover (transformed to percentage). Soil seed bank data are mean number of germinated seeds. Mean derived from sampling number of plots (N=10).

| Species  | Vegetation    |               | Soil seed bank  |                 |
|--|---------------|---------------|-----------------|-----------------|
|  | Pine clearing | Renoster-veld | Pine plantation | Pine Plantation |
| <b>in vegetation and soil seed bank</b>        |               |               |                 |                 |
| <i>Acacia saligna</i>                          | 0.50          |               |                 | 1.00            |
| <i>Aizoon sarmientosa</i>                      |               | 3.00          | 0.50            | 1.00            |
| <i>Anagallis arvensis</i> var. <i>caerulea</i> | 1.25          | 6.07          |                 | 8.44            |
| <i>Anthospermum hirtum</i>                     |               | 2.00          |                 | 1.33            |
| <i>Asphalatus flexuosa</i>                     | 1.21          | 0.50          |                 | 2.50            |
| <i>Athanasia trifurcata</i>                    | 0.50          | 15.00         |                 | 1.00            |
| <i>Avena barbata</i>                           | 15.00         |               | 16.63           | 7.00            |
| <i>Briza maxima</i>                            | 10.67         | 11.15         | 31.00           | 3.00            |
| <i>Briza minor</i>                             | 2.50          | 3.75          |                 | 11.80           |
| <i>Bromus pectinatus</i>                       |               | 8.50          |                 | 1.33            |
| <i>Conyza scabrada</i>                         |               |               |                 | 1.50            |
| <i>Cotula turbinata</i>                        | 3.00          |               |                 | 1.00            |
| <i>Diascia capensis</i>                        | 0.50          | 0.92          | 0.50            | 2.00            |
| <i>Erharhta longiflora</i>                     | 4.40          |               | 8.38            | 2.00            |
| <i>Fumaria muralis</i>                         | 1.13          | 0.10          |                 | 1.33            |
| <i>Helichrysum pandurifolium</i>               | 0.86          | 4.06          | 0.50            | 8.78            |
| <i>Helichrysum teretifolium</i>                |               | 2.06          |                 | 13.70           |
| <i>Hermannia alnifolia</i>                     |               | 3.00          |                 | 2.00            |
| <i>Lobelia erinus</i>                          | 0.50          |               |                 | 2.00            |
| <i>Medicago polymorpha</i>                     | 0.50          |               |                 | 1.00            |
| <i>Otholobium hirtum</i>                       | 0.86          | 0.10          |                 | 1.00            |
| <i>Oxalis pes-caprae</i>                       | 7.07          | 9.70          | 4.40            | 1.00            |
| <i>Picris echinoides</i>                       | 0.50          |               |                 | 2.00            |
| <i>Pseudognaphalium undulatum</i>              | 0.50          |               | 3.00            | 1.00            |
| <i>Sebaea aurea</i>                            | 0.50          | 1.07          | 0.50            | 3.80            |
| <i>Senecio hastatus</i>                        | 0.50          | 1.44          |                 | 1.00            |
| <i>Senecio pterophorus</i>                     | 1.75          |               |                 | 1.00            |
| <i>Senecio pubigerens</i>                      | 0.86          | 0.50          |                 | 1.17            |
| <i>Solanum guineense</i>                       |               | 3.00          | 0.30            | 1.00            |
| <i>Sonchus oleraceus</i>                       | 0.92          |               |                 | 1.71            |
| <i>Stachys aethiopica</i>                      |               | 3.00          | 2.17            | 1.00            |
| <i>Sutera uncinata</i>                         |               | 3.00          |                 | 2.29            |
| <i>Tribolium hispidum</i>                      | 0.50          |               |                 | 1.00            |
| <i>Tribolium uniolea</i>                       | 0.50          | 3.00          | 0.50            | 2.00            |
| <b>only in soil seed bank</b>                  |               |               |                 |                 |
| <i>Didymodoxa capensis</i>                     |               |               |                 | 2.00            |
| <i>Gastridium phleoides</i>                    |               |               |                 | 1.00            |
| geophyte 01                                    |               |               |                 | 4.50            |
| <i>Gnidia laxa</i>                             |               |               |                 | 1.00            |
| <i>Helichrysum asperum</i>                     |               |               |                 | 1.00            |

|                                   |      |       |       |      |
|-----------------------------------|------|-------|-------|------|
| <i>Helichrysum cymosum</i>        |      |       | 1.00  | 1.00 |
| <i>Helichrysum indicum</i>        |      |       | 1.40  |      |
| <i>Helichrysum luteo-album</i>    |      |       | 2.00  | 1.00 |
| herb 08                           |      |       | 6.00  |      |
| <i>Hypericum perforatum</i>       |      |       |       | 1.00 |
| <i>Isolepis marginata</i>         |      |       | 6.80  |      |
| <i>Lampranthus</i> sp.            |      |       |       | 1.00 |
| <i>Lolium multiflorum</i>         |      |       | 16.14 | 5.00 |
| <i>Nidorella foetida</i>          |      |       |       | 1.00 |
| <i>Ornithogalum graminifolium</i> |      |       |       | 1.33 |
| <i>Oxalis compressa</i>           |      |       | 2.00  |      |
| <i>Oxalis</i> sp.                 |      |       | 1.00  |      |
| <i>Phalaris minor</i>             |      |       | 1.00  | 1.00 |
| <i>Rhus</i> sp.                   |      |       |       | 1.00 |
| <i>Roellia ciliata</i>            |      |       | 1.00  |      |
| <i>Senecio burchelli</i>          |      |       | 4.50  |      |
| <i>Silene cretica</i>             |      |       |       | 1.00 |
| <i>Sonchus asper</i>              |      |       | 1.00  | 1.33 |
| <b>only in vegetation</b>         |      |       |       |      |
| <i>Berkheya armata</i>            | 0.50 | 2.50  | 0.50  |      |
| <i>Berkheya rigida</i>            | 0.50 | 0.50  | 0.50  |      |
| <i>Dimorphotheca pluvialis</i>    | 0.50 | 3.00  | 1.13  |      |
| <i>Erharta calycina</i>           | 3.90 | 7.00  | 1.75  |      |
| <i>Hesperantha falcata</i>        | 0.50 | 0.50  | 0.50  |      |
| <i>Oxalis purpurea</i>            | 1.75 | 6.56  | 1.75  |      |
| <i>Pterygodium catholicum</i>     | 0.50 | 1.42  | 0.50  |      |
| <i>Rhus laevigata</i>             | 0.50 | 1.65  | 0.86  |      |
| <i>Arcotheca calendula</i>        | 0.50 |       | 0.50  |      |
| <i>Asphalatus acanthophylla</i>   | 0.50 | 0.50  |       |      |
| <i>Bulbinella triquetra</i>       |      | 0.23  | 0.50  |      |
| <i>Cheilanthes capensis</i>       | 0.50 | 11.33 |       |      |
| <i>Cyphia digitata</i>            | 0.50 | 0.10  |       |      |
| <i>Drosanthemum hispidifolium</i> |      | 1.75  | 0.50  |      |
| <i>Echium plantagineum</i>        | 3.00 |       | 0.50  |      |
| <i>Euphorbia genistoides</i>      |      | 1.33  | 0.50  |      |
| <i>Gymnosporia buxifolia</i>      |      | 0.10  | 0.50  |      |
| <i>Hemimeris racemosa</i>         | 0.50 | 3.00  |       |      |
| <i>Lotononis prostrata</i>        | 0.50 | 6.17  |       |      |
| <i>Lycium afrum</i>               |      | 0.10  | 0.50  |      |
| <i>Moraea miniata</i>             | 0.42 |       | 0.10  |      |
| <i>Nemesia barbata</i>            | 0.50 | 0.50  |       |      |
| <i>Osteospermum spinosum</i>      |      | 0.50  | 0.50  |      |
| <i>Pelargonium myrrhifolium</i>   | 0.50 | 1.13  |       |      |
| <i>Pelargonium</i> sp. 02         | 5.33 |       | 3.88  |      |
| <i>Plantago lanceolata</i>        | 0.50 |       | 0.10  |      |
| <i>Reseda lutea</i>               | 4.75 | 3.00  |       |      |
| <i>Rhus tomentosa</i>             | 0.50 | 6.72  |       |      |
| <i>Salvia africana-lutea</i>      |      | 1.50  | 0.50  |      |
| shrub 04                          |      | 0.50  | 0.50  |      |
| <i>Sparaxis villosa</i>           |      | 0.50  | 0.42  |      |
| <i>Spiloxene capensis</i>         | 0.50 |       | 0.10  |      |
| <i>Spiloxene flaccida</i>         |      | 0.10  | 0.50  |      |
| <i>Tetragonia spicata</i>         |      | 3.00  | 4.36  |      |
| <i>Torilles arvensis</i>          | 0.50 | 1.75  |       |      |
| <i>Trachyandra muricata</i>       |      | 3.00  | 1.33  |      |

| Species                          | Pine clearing | Vegetation    |                 | Soil seed bank |               |
|----------------------------------|---------------|---------------|-----------------|----------------|---------------|
|                                  |               | Renoster-veld | Pine plantation | Pine clearing  | Renoster-veld |
| <i>Tulbaghia capensis</i>        |               | 3.00          | 0.50            |                |               |
| <i>Anisodonteia scabrosa</i>     | 0.50          |               |                 |                |               |
| <i>Anthospermum spathulatum</i>  |               | 15.19         |                 |                |               |
| <i>Arctopus echinatus</i>        |               | 15.00         |                 |                |               |
| <i>Asparagus capensis</i>        |               |               | 0.50            |                |               |
| <i>Asparagus sp.</i>             |               | 0.50          |                 |                |               |
| <i>Atriplex semibaccata</i>      |               |               | 3.00            |                |               |
| <i>Babiana stricta</i>           |               |               | 0.10            |                |               |
| <i>Baeometra uniflora</i>        | 0.50          |               |                 |                |               |
| <i>Bulbine sp.</i>               |               |               | 0.50            |                |               |
| <i>Chrysocoma ciliata</i>        | 0.50          |               |                 |                |               |
| <i>Cissampelos capensis</i>      |               | 0.10          |                 |                |               |
| <i>Cyphia phyteuma</i>           |               |               | 0.34            |                |               |
| <i>Dolichos decumbens</i>        |               |               | 0.50            |                |               |
| <i>Elytropappus rhinocerotis</i> |               | 25.30         |                 |                |               |
| <i>Empodium plicatum</i>         |               | 1.27          |                 |                |               |
| <i>Eriocephalus africanus</i>    |               | 9.20          |                 |                |               |
| <i>Erodium malacoides</i>        |               | 3.00          |                 |                |               |
| <i>Euphorbia erythrina</i>       | 0.50          |               |                 |                |               |
| <i>Felicia fructicosa</i>        |               | 4.13          |                 |                |               |
| <i>Festuca glabra</i>            |               |               | 3.00            |                |               |
| <i>Ficinia indica</i>            | 1.33          |               |                 |                |               |
| geophyte 02                      |               |               | 0.50            |                |               |
| geophyte 03                      |               |               | 1.33            |                |               |
| geophyte 06                      |               | 1.75          |                 |                |               |
| geophyte 07                      |               | 6.17          |                 |                |               |
| geophyte 08                      |               | 1.92          |                 |                |               |
| geophyte 09                      |               | 0.50          |                 |                |               |
| geophyte 10                      |               | 0.50          |                 |                |               |
| geophyte 11                      |               | 0.50          |                 |                |               |
| geophyte 12                      |               | 0.50          |                 |                |               |
| geophyte 13                      |               | 3.00          |                 |                |               |
| <i>Geranium canescens</i>        |               | 0.30          |                 |                |               |
| <i>Geranium molle</i>            |               | 2.58          |                 |                |               |
| gras 03                          | 0.50          |               |                 |                |               |
| gras 04                          | 0.50          |               |                 |                |               |
| <i>Helichrysum revolutum</i>     |               | 1.93          |                 |                |               |
| herb 01                          |               |               | 0.50            |                |               |
| herb 02                          |               | 0.10          |                 |                |               |
| <i>Hermania hyssopifolia</i>     | 0.50          |               |                 |                |               |
| <i>Hermannia rugosa</i>          | 0.50          |               |                 |                |               |
| <i>Hesperantha radiata</i>       |               | 0.50          |                 |                |               |
| <i>Hypochoeris radicata</i>      | 0.50          |               |                 |                |               |
| <i>Indigofera sp.</i>            | 0.50          |               |                 |                |               |
| <i>Indigofera sp.2</i>           |               | 2.17          |                 |                |               |
| <i>Lampranthus emarginatus</i>   |               | 0.50          |                 |                |               |
| <i>Leysera gnaphalodes</i>       |               | 0.50          |                 |                |               |
| <i>Montinia caryophyllaceae</i>  |               | 3.00          |                 |                |               |
| <i>Morea bellendenii</i>         | 1.33          |               |                 |                |               |
| <i>Morea gawleri</i>             | 0.50          |               |                 |                |               |
| <i>Morea sp.</i>                 |               | 3.00          |                 |                |               |
| <i>Morea sp. 2</i>               |               |               | 1.75            |                |               |
| <i>Muraltia ononidifolia</i>     |               |               | 0.50            |                |               |

|                                      |      |       |      |      |  |
|--------------------------------------|------|-------|------|------|--|
| <i>Olea europaea subsp. africana</i> |      | 0.50  |      |      |  |
| <i>Ornithogalum thyrsoides</i>       |      | 0.50  |      |      |  |
| <i>Oxalis argyrophylla</i>           | 2.17 |       |      |      |  |
| <i>Oxalis lanata</i>                 |      | 3.00  |      |      |  |
| <i>Oxalis tomentosa</i>              |      | 1.33  |      |      |  |
| <i>Oxalis versicolour</i>            | 0.50 |       |      |      |  |
| <i>Pelargonium cucullatum</i>        | 0.50 |       |      |      |  |
| <i>Pelargonium sp.01</i>             | 0.50 |       |      |      |  |
| <i>Pinus sp.</i>                     | 0.50 |       |      |      |  |
| <i>Pterona hirsuta</i>               |      | 0.50  |      |      |  |
| <i>Rapistrum rugosum</i>             |      | 0.50  |      |      |  |
| <i>Romulea sp.</i>                   |      | 0.10  |      |      |  |
| <i>Salvia africana-caerulea</i>      |      | 0.50  |      |      |  |
| <i>Satyrium odorum</i>               |      | 3.00  |      |      |  |
| <i>Scabiosa columbaria</i>           |      | 0.10  |      |      |  |
| <i>Sebaea exacoides</i>              | 1.33 |       |      |      |  |
| <i>Senecio pubigerens</i>            |      | 0.50  |      |      |  |
| shrub 02                             |      |       | 0.50 |      |  |
| shrub 05                             |      |       | 3.00 |      |  |
| shrub 06                             |      |       | 3.00 |      |  |
| <i>Silene undulata</i>               |      |       |      | 0.50 |  |
| <i>Sparaxis glandiflora</i>          | 0.50 |       |      |      |  |
| <i>Themeda triandra</i>              |      | 15.00 |      |      |  |
| <i>Trachyantra hirsutiflora</i>      |      |       |      | 0.50 |  |
| <i>Vicia sativa</i>                  |      |       |      | 0.50 |  |
| <i>Viscum capense</i>                |      | 0.10  |      |      |  |
| <i>Zantedeschia aethopica</i>        |      | 1.55  |      |      |  |

## 6 *Hemimeris racemosa* populations sustain high genetic variation in the fragmented renosterveld of South Africa

### Abstract

Anthropogenic land use change results in fragmentation of plant populations thereby reducing population sizes and increasing population distances. This habitat fragmentation may be responsible for the decrease of genetic variation in remnant plant populations and higher genetic variation between them. Renosterveld vegetation of South Africa is a highly fragmented and endangered Mediterranean-type shrubland. Prior to transformation, species-rich renosterveld filled large proportions of the lowlands in the south-western Cape, leaving only ten percent in small remnants. Three regional patterns are common in this fragmented landscape with a varying degree of remnant size and isolation: large, medium-distant remnants at Tygerberg area; semi-large, small-distant remnants at Botteleray Hills; and small, large-distant remnants at Swartland. In each region, the annual herb *Hemimeris racemosa* (Scrophulariaceae) was examined in six, seven and twelve populations, respectively. It was argued that fragmentation of its endangered renosterveld habitat could have a negative effect on general genetic variation and might be influenced by fragmentation degree. Despite the long fragmentation history, AFLP analysis revealed considerable genetic variation within (Nei's gene diversity=0.18) and low genetic variation between populations ( $\Phi_{ST}$ =0.09). Furthermore, low differences of genetic variation between populations (mean  $\Phi_{ST}$ =0.05) were detected when the three fragmentation regions were analyzed separately. From the data it can be assumed that fragmentation and fragmentation degree play a minor role in the genetic structuring of *H. racemosa*. Sufficient historical and possibly recent gene flow might mitigate the results of habitat fragmentation.

## Introduction

Renosterveld vegetation of South Africa is a highly fragmented and endangered shrubland occurring in Mediterranean-type climate and on nutrient-rich soils (Rebelo *et al.* 2006). Negative impacts on plant-pollinator mutualism were observed in small conservation areas, resulting in reduced pollination, seed set and genetic diversity (Pauw *et al.* 2004, Pauw 2007). Currently, the latter studies are the only dealing with the effects of fragmentation on genetic variation in renosterveld.

On the background of globally accelerating habitat loss and fragmentation (Saunders *et al.* 1991), landscape genetics have become an important research field (Manel and Segelbacher 2009). Molecular techniques enable the analysis of genetic variation in species and allow setting conservation goals in order to maintain genetic variation of fragmented populations. This may avoid inbreeding depressions (Saccheri *et al.* 1998), ensure evolutionary processes (Lande and Barrowclough 1987) and enhance species persistence in a global change scenario (Hedrick and Miller 1992). In general, ecological and genetical processes interact and influence genetic variation of species that underpins short-term fitness (Huenneke *et al.* 1991), long-term survival and population persistence (Lee *et al.* 2006).

Habitat fragmentation is an anthropogenic induced process where land use leads to transformation of pristine habitats thereby resulting in fragmentation of plant populations (Harrison and Bruna 1999). On the one hand, it will lead to habitat loss, reduced habitat size (Luijten *et al.* 2000), and smaller population sizes (Oostermeijer *et al.* 1996, Bruna and Kress 2002, Van Rossum *et al.* 2004). On the other hand, it will increase separation, isolation and interior-to-edge ratio of populations (Franklin *et al.* 2002). These parameters negatively affect biota, such as species richness (Turner 1996), number of specialists (Kruess and Tscharntke 1994), and stages of the life cycle (Amler *et al.* 1999). Furthermore, habitat fragmentation may result in reduced gene flow (Ouborg *et al.* 2006), loss of genetic variation (Fahrig 2001, Honnay *et al.* 2006), inbreeding and drift (Young *et al.* 1996), and the decrease of short- and long-term population viability (Ellstrand and Elam 1993).

Genetic variation is the variation in alleles of genes and occurs within and between populations (Linhart *et al.* 1981, Schmitt and Gamble 1990), as well as in the entire species range (Li and Adams 1989). Genetic variation is essential for natural selection processes and influenced by the magnitude of fragmentation, as well as ecological and genetical factors. Species rarity is such a parameter and in general, higher genetic variation occurs in common species. Although common species are less prone to habitat fragmentation effects (Bijlsma and Loeschcke 2005) it does not seem to have an effect on population differentiation

(Hamrick and Godt 1989, Gitzendanner and Soltis 2000, Cole 2003). Genetic variability strongly depends on plant functional traits of the particular species. Higher genetic variability within populations is found in long-lived, woody, outcrossing and late-successional species compared to short-lived, non-woody, self-compatible, and early-successional species, which inherit higher genetic variation between populations (Hamrick and Godt 1996, Nybom and Bartish 2000). In general, fragmentation induced reduction of population size is responsible for the decrease of genetic variation rather than habitat differences (Fischer and Matthies 1998). Many studies deal with population size and genetic variation (Oostermeijer *et al.* 2003) and it seems that the relation is positive and more stronger in self-incompatible than self-compatible plants (Leimu *et al.* 2006). Small populations with less than 100 individuals are more prone to genetic drift than larger populations with over 1000 individuals; here genetic drift becomes a minor factor (Montalvo *et al.* 1997). While most studies have looked at population size effects, few studies have examined whether increasing population distance affects genetic variation between plant population remnants. However, the need to incorporate population isolation as a parameter is stressed in order to evaluate the spatial genetic structure (Ouborg *et al.* 2006). Both, negative effects (Schmidt and Jensen 2000, Lienert *et al.* 2002) and neutral effects (Foré *et al.* 1992, Young *et al.* 1993) can be observed.

Continuously populated habitats allow gene flow by means of pollen and diaspores, enabling processes of adaptation and evolution (Bishop 1972, Heywood 1991). Fragmentation leads to reduced gene flow and possibly negative impacts on genetic variation (Young *et al.* 1996, Landergott *et al.* 2001). Gene flow pattern in naturally fragmented plant populations are well studied (Larson *et al.* 1984, Ellstrand and Marshall 1985) concluding that such species are relatively prone to inbreeding depression (Huenneke *et al.* 1991). However, little is known about these processes in anthropogenic-caused fragmentation (Lacy 1987, Lande and Barrowclough 1987, Robinson and Quinn 1992, Montalvo *et al.* 1997), where viability of remnant populations might be uncertain and critical (Tansley 1988).

Prior to transformation, species-rich renosterveld filled large proportions of the lowlands in the south-western Cape (Kemper *et al.* 1999). Nowadays, only ten percent of the former extent occurs in isolated remnants (von Hase *et al.* 2003a). Geomorphologic features, such as hills, hill ridges and lowland, coupled with a varying extent of agricultural land conversion resulted in three common fragmentation regions that show a varying degree of remnant size and isolation: large remnants with medium-distant at the Tygerberg area (region A), semi-large remnants with small-distant at the Botteleray Hills (region B), and small remnants with large-distant to neighbour at the Swartland (region C). This fragmentation situation allows

comparing less fragmented region A and B with region C that show a hypothetical result of further habitat loss and isolation. Such situation is known as space-for-time substitution (Pickett 1989, Hargrove and Pickering 1992, Travis and Hester 2005, Honnay *et al.* 2009).

The annual herb *Hemimeris racemosa* (Scrophulariaceae) is outcrossing, oil-secreting, and pollinated by several species of oil-collecting bees (Steiner and Whitehead 2002, Pauw 2004) and occurs in all three fragmentation regions. Within this setting, detection of strong fragmentation effect on genetic variation of *H. racemosa* populations and fragmentation regions was hypothesized. Concerning general genetic structure, it was suggested to find low genetic variation within and high genetic variation between populations. The population genetic structure within the three fragmentation regions A, B and C, should inherit gradual genetic variation, depending on fragment size and isolation.

## Material and Methods

### *Species description*

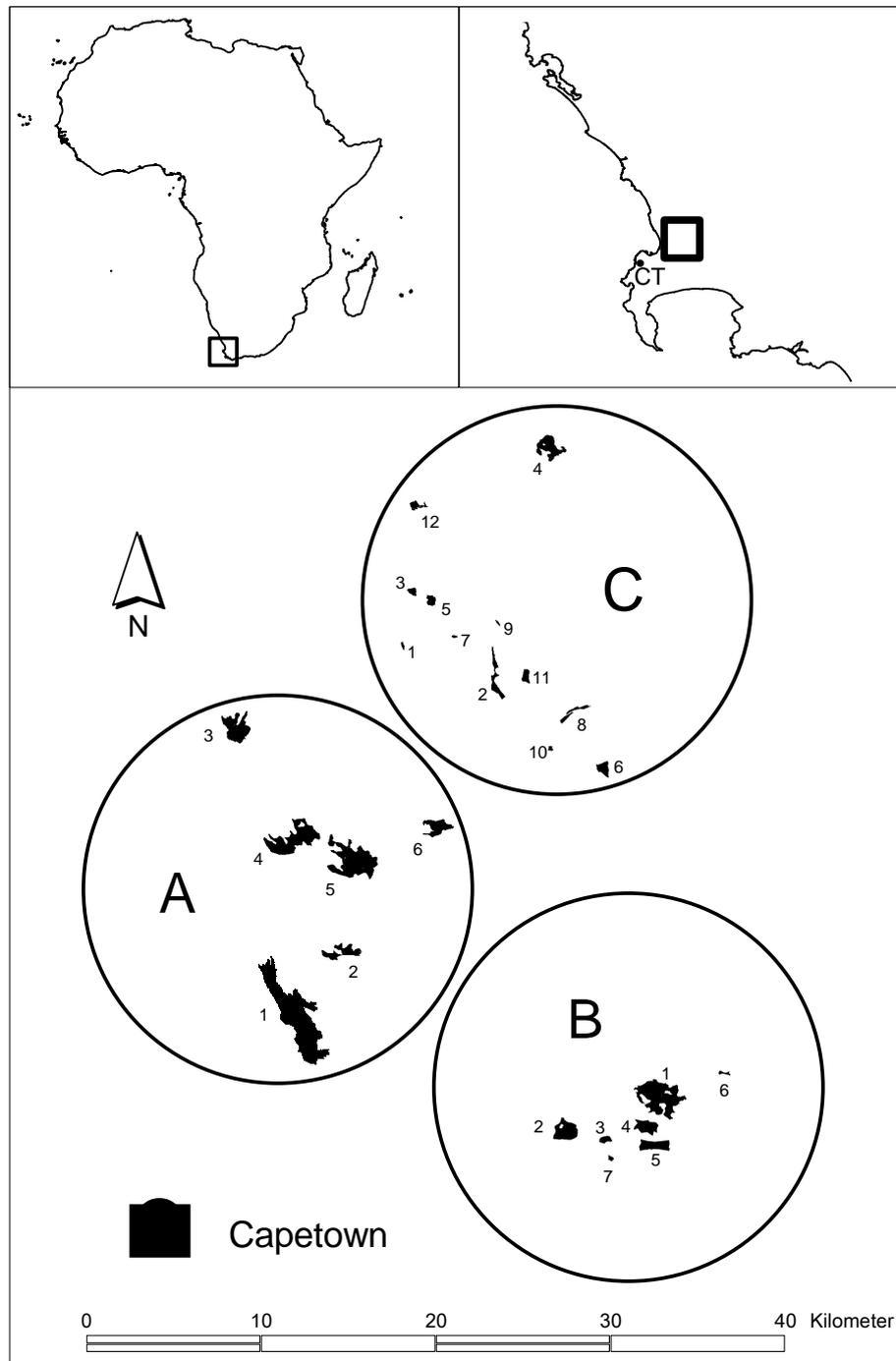
*Hemimeris racemosa* (Houtt.) Merrill is classified in the tribe Hemimerideae of the Scrophulariaceae family (Olmstead *et al.* 2001). It is limited to the Southern hemisphere and widespread throughout the Cape Floristic Region of South Africa found in high abundances on sand and clay soils (Goldblatt and Manning 2000). This annual herb is diploid and has a chromosome number of  $x=14$  (Steiner 1996). *H. racemosa* grows up to 40 cm in height (Fig. 20) and has ovate and toothed leaves (Goldblatt and Manning 2000). It flowers from July to October and is characterized by double spurred and axillary yellow flowers between 7-13 mm in diameter. Furthermore, a stilar polymorphism is described (Pauw 2005). *H. racemosa* is an outcrossing and oil-secreting specialist, pollinated by several species of oil-collecting female bees of *Rediviva* spp. and pollen-eating beetles of the tribes Nitidulidae, Melyridae and Scarabaeidae (Whitehead and Steiner 2001, Pauw 2004, 2005). Seeds are small and gravity-dispersed.



**Fig. 20. Habitus of *Hemimeris racemosa***

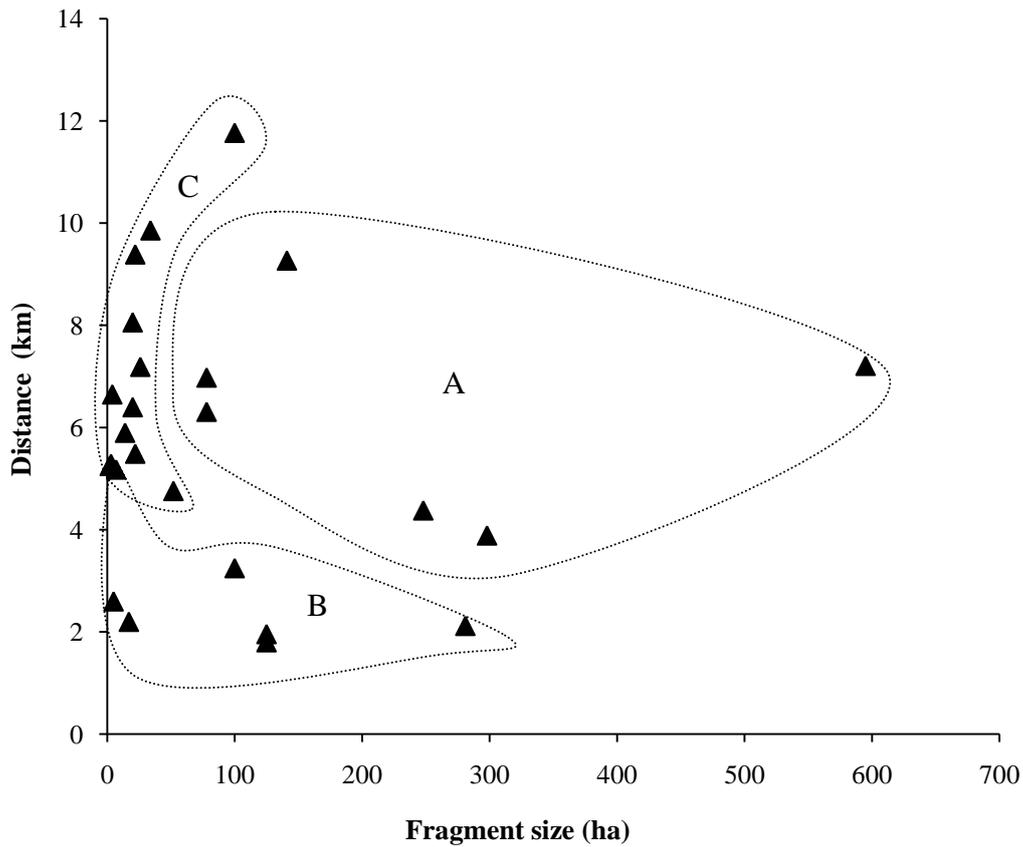
#### *Sampling procedure and landscape setup*

The study area comprises the Cape lowlands within the West Coast Centre renosterveld (Rebello *et al.* 2006), situated up to 40 kilometres north and east of Cape Town. The sampling was performed in three common fragmentation regions covering virtually all available fragments within an 11 km radius (Fig. 21). It was possible to study genetic variation of 25 *H. racemosa* populations (Table 11), where fragment size was estimated as population size. For each of the fragments, mean distance to neighbour was determined in GIS (ArcView3.2, ESRI 2000). Mean distance was calculated as an index resulting from the mean value of each edge-to-edge distance from one particular fragment to all other fragments. This index was made available for the entire dataset, and done separately for each fragmentation region. Each fragmentation region is distinguished by different sizes and neighbouring distance and shows therefore a particular grouping (Fig. 22). The following fragmentation regions are present in the Cape Lowlands: large remnants with medium-distant (region A), semi-large remnants with small-distant (region B), and small remnants with large-distant (region C).



**Fig. 21. Sampling sites in different fragmentation regions of renosterveld.**

Circles (diameter 22 km) and letters indicate fragmentation regions with sampling sites. Cape Town (CT). For details see Table 11.



**Fig. 22. Sampling sites with fragment size and mean distance to neighbour fragment.** Fragmentation regions A, B and C are encircled and indicated by different letters. Details in Table 11.

#### *DNA isolation and AFLP analysis*

For each population leaf material of up to 20 individuals was collected and cooled on ice. Later they were placed into filter bags and dehydrated in silica gel. DNA was isolated from 10 mg of dried plant material of individual plants using CTAB method (cetyltrimethylammonium bromide, Rogers and Bendich 1994). Both, DNA isolation and AFLP method (Vos *et al.* 1995) were adapted as previously described (Reisch *et al.* 2005, Reisch 2008). DNA concentration was photometrical estimated and samples were standardised at a dilution of 7.8 ng/ $\mu$ l. For the AFLP procedure, genomic DNA (approximately 50 ng) was used for restriction and ligation reaction with MseI and EcoRI restriction enzymes and T4 DNA Ligase (both Fermentas) conducted in a thermal cycler for 2 h at 37 °C. Polymerase chain reactions (PCRs) were run in a reaction volume of 5 mL. Preselective amplifications were performed using primer pairs with a single selective nucleotide, MseI and EcoRI together with H<sub>2</sub>O, Puffer S, dNTPs and Taq-Polymerase (PeqLab). The PCR reaction parameters were: 2 min at 94 °C, 30 cycles of 20 sec of denaturing at 94 °C, 30 sec of annealing at 56 °C, and 2 min of extension at 72 °C, followed

by 2 min at 72 °C and ending with 30 min at 60 °C. After an extensive screening of selective primer combinations with eight randomly selected samples, selective amplifications were performed with the three primer combinations (MseI+CTC/EcoRI+AAC, MseI+CTC/EcoRI+AAG, MseI+CTG/EcoRI+ACT) and H<sub>2</sub>O, dNTPs and Taq-Polymerase (PeqLab).

**Table 11. Sampled populations of *Hemimeris racemosa*.**

Bold numbers show mean values of each category. † Data following von Hase *et al.* (2003a) and estimations.

\* Data are extracted from aerial photographs.

| Fragmentation region and population | Longitude (E)       | Latitude (S) | Fragment size (ha)†  | Mean distance to neighboring remnants (km) ± S.E |              |
|-------------------------------------|---------------------|--------------|----------------------|--|--------------|
| A                                   | 1 Tygerberg         | 18°35'39"    | 33°52'37"            | 595  | 7.21 ± 1.45  |
|                                     | 2 Kanonkop          | 18°36'16"    | 33°49'35"            | 78   | 6.31 ± 1.43  |
|                                     | 3 Koeberg           | 18°33'28"    | 33°42'49"            | 141  | 9.27 ± 1.19  |
|                                     | 4 Porquepine        | 18°35'15"    | 33°46'10"            | 248  | 4.38 ± 0.81  |
|                                     | 5 Meerendal         | 18°37'23"    | 33°46'59"            | 298  | 3.89 ± 0.92  |
|                                     | 6 Sondagsfontein    | 18°39'44"    | 33°45'50"            | 78   | 6.98 ± 1.18  |
|                                     |                     |              | <b>239.66 ± 79.9</b> | <b>6.34 ± 0.81</b>                               |              |
| B                                   | 1 Koopman. PNR      | 18°45'55"    | 33°54'14"            | 281  | 2.12 ± 0.44  |
|                                     | 2 Zevenwacht        | 18°43'35"    | 33°55'16"            | 100  | 3.25 ± 0.90  |
|                                     | 3 Mooiplaas         | 18°44'32"    | 33°55'29"            | 17   | 2.20 ± 0.85  |
|                                     | 4 Wolfkloof bottom* | 18°45'58"    | 33°54'53"            | 125  | 1.80 ± 0.58  |
|                                     | 5 Wolfkloof top*    | 18°46'15"    | 33°55'17"            | 125  | 1.96 ± 0.60  |
|                                     | 6 Koopman. East*    | 18°46'58"    | 33°54'04"            | 7  | 5.18 ± 0.83  |
|                                     | 7 Morgenzon         | 18°45'11"    | 33°55'50"            | 5  | 2.60 ± 0.92  |
|                                     |                     |              | <b>94.28 ± 37.3</b>  | <b>2.73 ± 0.45</b>                               |              |
| C                                   | 1 Middlepos         | 18°38'37"    | 33°40'14"            | 4  | 6.65 ± 1.11  |
|                                     | 2 Klipheuwel        | 18°41'23"    | 33°41'52"            | 52   | 4.76 ± 1.12  |
|                                     | 3 Remshoogte S      | 18°38'55"    | 33°38'33"            | 20   | 6.40 ± 1.19  |
|                                     | 4 Helderfontein     | 18°42'52"    | 33°34'03"            | 100  | 11.77 ± 0.96 |
|                                     | 5 Remshoogte L*     | 18°39'29"    | 33°38'51"            | 14   | 5.90 ± 1.19  |
|                                     | 6 Klapmuts          | 18°44'45"    | 33°44'04"            | 34   | 9.86 ± 1.60  |
|                                     | 7 Bonnie Doon       | 18°40'13"    | 33°39'56"            | 3  | 5.29 ± 1.05  |
|                                     | 8 Bloublomme        | 18°43'37"    | 33°42'27"            | 26   | 7.19 ± 1.39  |
|                                     | 9 Wintervogel       | 18°41'32"    | 33°39'29"            | 2  | 5.25 ± 0.89  |
|                                     | 10 Kliprug*         | 18°43'07"    | 33°43'19"            | 20   | 8.06 ± 1.57  |
|                                     | 11 Area 61          | 18°42'24"    | 33°40'54"            | 22   | 5.49 ± 0.99  |
|                                     | 12 Uitspan          | 18°38'49"    | 33°35'30"            | 22   | 9.39 ± 1.26  |
|                                     |                     |              | <b>26.58 ± 7.8</b>   | <b>7.17 ± 0.63</b>                               |              |

PCR reactions were performed with the touch-down profile: 2 min at 94 °C, ten cycles of 20 sec of denaturing at 94 °C, 30 sec of annealing, which was initiated at 66 °C and then reduced by 1 °C for the next ten cycles, 2 min of elongation at 72 °C, followed by 25 cycles of 20 s of denaturing at 94 °C, 30 sec of annealing at 56 °C and 2 min of elongation at 72 °C, ending with a final extension for 30 min at 60 °C. After DNA precipitation, DNA pellets were vacuum-dried and dissolved in a mixture of Sample Loading Solution and CEQ Size Standard 400 (both Beckman Coulter). The fluorescence-labelled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter). Raw data were collected and analysed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter). Data were exported as crv-files, showing synthetic gels with AFLP fragments for each primer combination separately from all studied individuals and analysed in BIONUMERICS (Applied Maths, v. 3.0). Files were examined for strong, clearly defined bands. Each band was scored across all individuals as either present or absent.

#### *Statistical analysis*

In the AFLP data matrix, the presence of a band was scored as 1, whereas the absence of the band was coded as 0. Finally, basic data structure consisted of a binomial (0/1) matrix, representing the scored AFLP markers. Nei's Gene Diversity, Shannon's Information Index, and number and percentage of polymorphic loci (PL) were calculated for each population and fragmentation region. Genetic variation were calculated via POPGENE v. 1.32 (Yeh *et al.* 1999) and tested for differences using Kruskal-Wallis *H*-test and Mann-Whitney *U*-test. Genetic variation within populations was calculated based on polymorphic bands as Nei's gene diversity [ $GD = \sum_i h_{ij}/I$ ] (Nei 1978) and Shannon Information Index [ $SI = \sum p_i \ln p_i$ ] (Lewinton 1972) for each population. The binomial matrix was subjected to an analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) using GENALEX v. 6.2 (Peakall and Smouse 2006). Variance components and their significance levels for variation among regions, among populations and within populations were calculated.  $F_{st}$  approximately equals  $G_{st}$  and are used for comparisons of species differentiation levels (Hartl and Clark 1989). Bayesian analysis was applied by using STRUCTURE v. 2.2 (Pritchard *et al.* 2000) to determine number of group assignment (Evanno *et al.* 2005). Allele frequencies were used as uncorrelated in an admixture model.  $10^4$  iterations for estimating the number of groups with at burn-in-period of  $10^4$  were applied. For each predefined number of *K* (2-27) 10 iterations were run. From the resulting values of  $L(K)$  standard deviations and  $\Delta K$  were calculated to

reveal the most likely number of groups (Evanno *et al.* 2005). Furthermore, group assignment of all individuals was visualized via a bar plot. Genetic relatedness between individuals, assorting populations and differences between the three regions was analysed by principle coordinates analysis (PcoA). Calculations and plotting was based on inter-individual Bray-Curtis similarities and performed in MVSP v. 3.12 (Kovach 1999). A Mantel test, based on 999 permutations, was conducted to test whether the matrix of pair-wise genetic distances ( $\Phi_{PT}$ ), taken from the AMOVA between populations, was correlated with the matrix of geographical distances between populations (Mantel 1967, Sokal and Rohlf 1995).

## Results

### *General genetic structure*

AFLP analyses revealed 272 fragments (MseI+CTC/EcoRI+AAC [85 fragments], MseI+CTC/EcoRI+AAG [91 fragments], MseI+CTG/EcoRI+ACT [96 fragments]) with 66.9 % mean polymorphic loci, ranging from 47.8 to 79.8 % (Table 12). Mean Nei's gene diversity was 0.18, ranging from 0.14 to 0.21. Mean Shannon's information index was 0.28, ranging from 0.21 to 0.32. Lowest genetic variation was found at population B-4 (GD=0.14, SI=0.21, PL=48 %) and highest genetic variation was in population A-4 (GD=0.21), A-4 and C-7 (SI=0.32) and A-2 (PL=80 %). No significant correlation of fragment size and distance with genetic variation was visible (Table 13). Analysis of molecular variances (two-level AMOVA, 9 %,  $\Phi_{PT}$ =0.09) showed low genetic variation between populations (Table 14).

**Table 12. Genetic variation within populations of *Hemimeris racemosa*.**

Fragmentation region according to Table 11. Sampling size (N). Bold numbers show mean values of each fragmentation region.

| Fragmentation region and population number | N | Nei's gene diversity (GD ± S.E.) | Shannon's index (SI ± S.E.) | Percentage of polymorphic loci (PL) |
|--|---|----------------------------------|-----------------------------|-------------------------------------|
| A  | 1 | 0.17 ± 0.01                      | 0.26 ± 0.02                 | 61.8                                |
|  | 2 | 0.18 ± 0.01                      | 0.28 ± 0.02                 | 68.4                                |
|  | 3 | 0.18 ± 0.01                      | 0.29 ± 0.01                 | 71.3                                |
|  | 4 | 0.21 ± 0.01                      | 0.32 ± 0.02                 | 76.8                                |
|  | 5 | 0.18 ± 0.01                      | 0.28 ± 0.01                 | 73.5                                |
|  | 6 | 0.15 ± 0.01                      | 0.24 ± 0.02                 | 56.3                                |

**Table 12 cont.**

|                 |    |    |                    |                    |                    |
|-----------------|----|----|--------------------|--------------------|--------------------|
| B               | 1  | 20 | 0.17 ± 0.01        | 0.27 ± 0.02        | 64.3               |
|                 | 2  | 19 | 0.18 ± 0.01        | 0.28 ± 0.02        | 72.2               |
|                 | 3  | 20 | 0.16 ± 0.01        | 0.25 ± 0.02        | 64.0               |
|                 | 4  | 10 | 0.14 ± 0.01        | 0.21 ± 0.02        | 47.8               |
|                 | 5  | 14 | 0.16 ± 0.01        | 0.25 ± 0.02        | 60.3               |
|                 | 6  | 13 | 0.17 ± 0.01        | 0.26 ± 0.02        | 61.81              |
|                 | 7  | 6  | 0.16 ± 0.01        | 0.24 ± 0.02        | 48.2               |
| C               | 1  | 19 | 0.19 ± 0.01        | 0.30 ± 0.01        | 73.9               |
|                 | 2  | 20 | 0.19 ± 0.01        | 0.30 ± 0.01        | 79.8               |
|                 | 3  | 18 | 0.18 ± 0.01        | 0.28 ± 0.01        | 70.2               |
|                 | 4  | 8  | 0.16 ± 0.01        | 0.25 ± 0.02        | 53.0               |
|                 | 5  | 18 | 0.19 ± 0.01        | 0.29 ± 0.02        | 71.3               |
|                 | 6  | 18 | 0.18 ± 0.01        | 0.28 ± 0.01        | 69.9               |
|                 | 7  | 20 | 0.20 ± 0.01        | 0.32 ± 0.01        | 77.2               |
|                 | 8  | 12 | 0.17 ± 0.01        | 0.27 ± 0.02        | 65.4               |
|                 | 9  | 19 | 0.18 ± 0.01        | 0.28 ± 0.01        | 73.2               |
|                 | 10 | 12 | 0.18 ± 0.01        | 0.29 ± 0.02        | 66.9               |
|                 | 11 | 17 | 0.18 ± 0.01        | 0.29 ± 0.02        | 71.0               |
|                 | 12 | 20 | 0.18 ± 0.01        | 0.29 ± 0.01        | 76.1               |
| All populations |    |    | <b>0.18 ± 0.01</b> | <b>0.28 ± 0.01</b> | <b>66.9 ± 1.74</b> |

**Table 13. Pearson correlation coefficient of fragment size and distance with genetic variation.** Significant correlations did not occur ( $p < 0.05$ ).

|                 | Correlation of fragment size with |        |        | Correlation of mean distance to neighboring remnants with |       |       |
|-----------------|-----------------------------------|--------|--------|---|-------|-------|
|                 | GD                                | SI     | PL     | GD  | SI    | PL    |
| All populations | -0.051                            | -0.103 | -0.094 | 0.175   | 0.253 | 0.154 |

**Table 14. Analysis of molecular variance of *Hemimeris racemosa*.**

Based on 272 AFLP fragments. Proportion of genetic variation (%). Significance level ( $p < 0.001$ ) is based on 999 permutations.

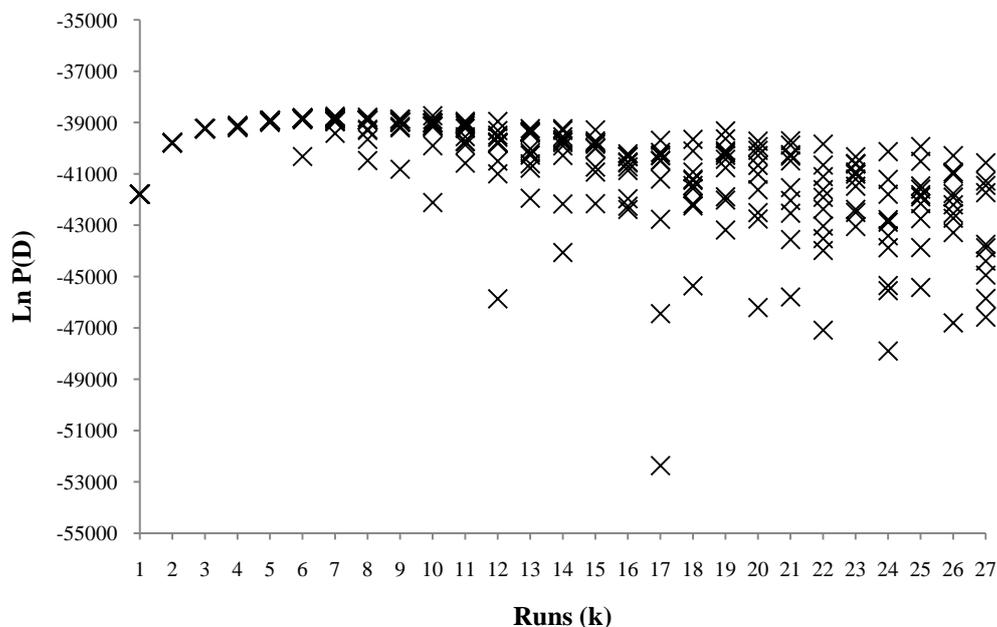
| Individuals | Populations | Genetic variation   | Degrees of freedom | Sums of squares | Means squares | %   | Phi <sub>Pt</sub> |
|-------------|-------------|---------------------|--------------------|-----------------|---------------|-----|-------------------|
| 398         | 25          | Between populations | 24                 | 1902.70         | 79.28         | 9%  | 0.09              |
|             |             | Within populations  | 373                | 11208.76        | 30.05         | 91% |                   |

Bayesian analysis revealed two groups as the most likely number of groupings for the entire dataset (Table 15, Fig. 23, Fig. 24). The bar plot shows group allocation for each individual and fragmentation region B as a distinct group (Fig. 25). Principal coordinates analysis of entire data set revealed two groupings (Fig. 26). Mantel test revealed significant isolation by distance of the entire dataset (Fig. 27).

**Table 15. Bayesian model-based clustering likelihoods and model selection.**

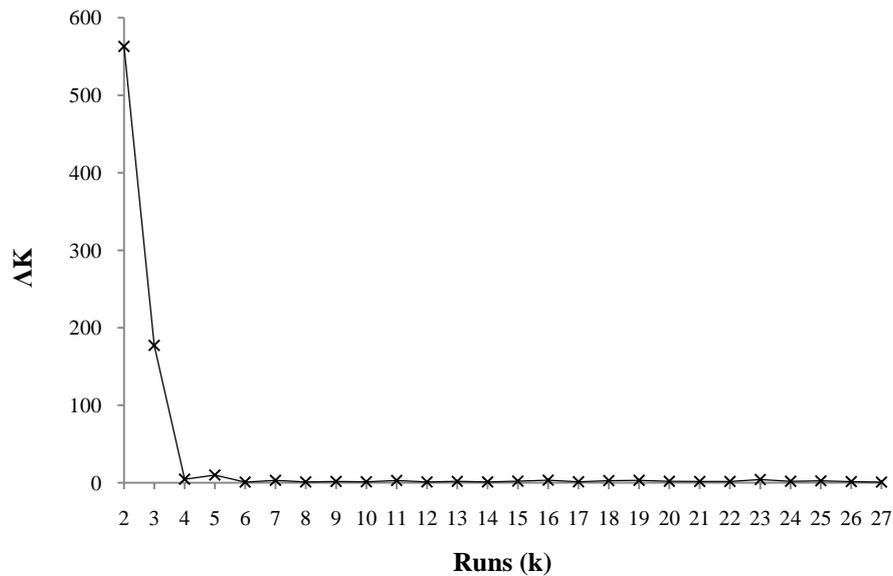
True number of groups (k) and log probability of data Ln P(D). Ad hoc quantity based on rate of change of likelihood function with respect to K calculation ( $\Delta K$ ). 10 replicate runs. Values of  $K > 10$  are not reported due to low likelihood. Model selection for true number of groups (k) present in the dataset. ‡Model selection method (Evanno *et al.* 2005).

| k  | Mean ln P(D) | S.E.   | Variance  | S.D.   | $\Delta k$ ‡  |
|----|--------------|--------|-----------|--------|---------------|
| 1  | -41792.18    | 0.19   | 0.35      | 0.59   |               |
| 2  | -39785.67    | 0.82   | 6.74      | 2.60   | <b>563.18</b> |
| 3  | -39240.78    | 0.81   | 6.54      | 2.56   | 177.24        |
| 4  | -39149.30    | 8.31   | 691.05    | 26.29  | 4.71          |
| 5  | -38935.49    | 8.78   | 770.42    | 27.76  | 10.05         |
| 6  | -38992.37    | 148.26 | 219809.34 | 468.84 | 0.91          |
| 7  | -38923.20    | 59.49  | 35388.47  | 188.12 | 3.21          |
| 8  | -39195.02    | 167.50 | 280553.58 | 529.67 | 1.19          |
| 9  | -39172.36    | 186.68 | 348487.18 | 590.33 | 1.73          |
| 10 | -39413.76    | 315.78 | 997170.17 | 998.58 | 1.35          |



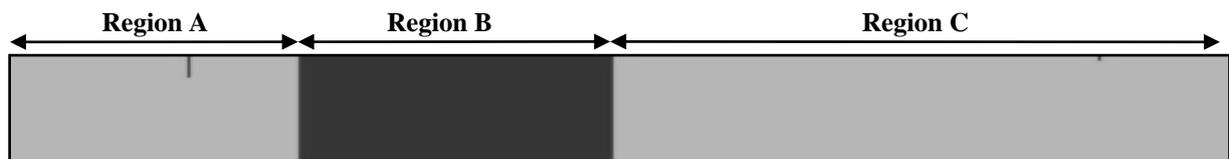
**Fig. 23. Bayesian analysis of group allocation for *Hemimeris racemosa*.**

True number of groups (k) and log probability of data Ln P(D). Calculation based on 272 AFLP fragments for 398 *Hemimeris racemosa* individuals from 25 populations using logarithmic likelihood. 10 replicates runs revealed the most likely number of groups  $K=2$  with lowest variance of Ln P is S.D.=2.6.

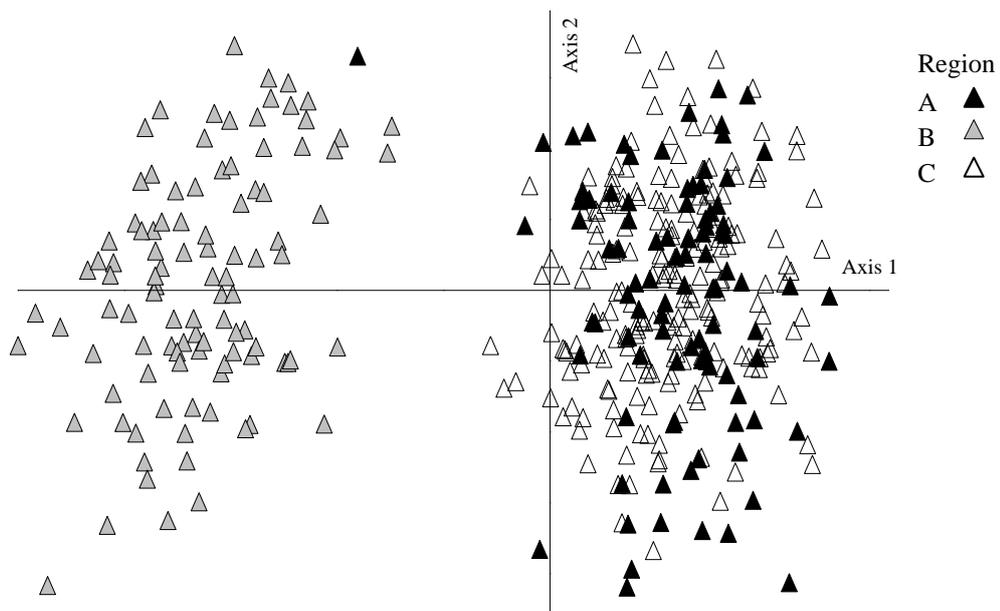


**Fig. 24. Bayesian analysis of group allocation for *Hemimeris racemosa*.**

True number of groups ( $k$ ). Ad hoc quantity based on rate of change of likelihood function with respect to  $K$  calculation ( $\Delta K$ ). Based on 272 AFLP fragments for 398 *Hemimeris racemosa* individuals from 25 populations using logarithmic likelihood. 10 replicates runs revealed the most likely number of groups  $K=2$  with highest  $\Delta K=563.2$ .

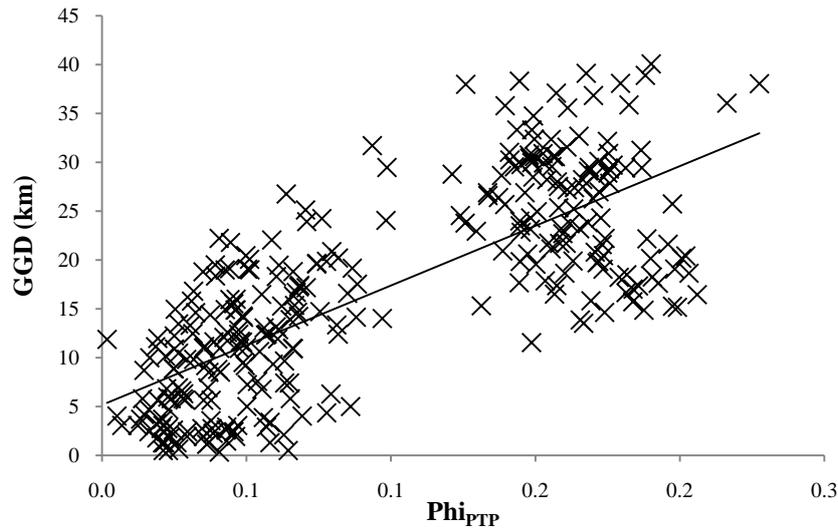


**Fig. 25. Assignments of *Hemimeris racemosa* individuals to  $K=2$  demes.**



**Fig. 26. Principal coordinates analysis of 398 *Hemimeris racemosa* individuals.**

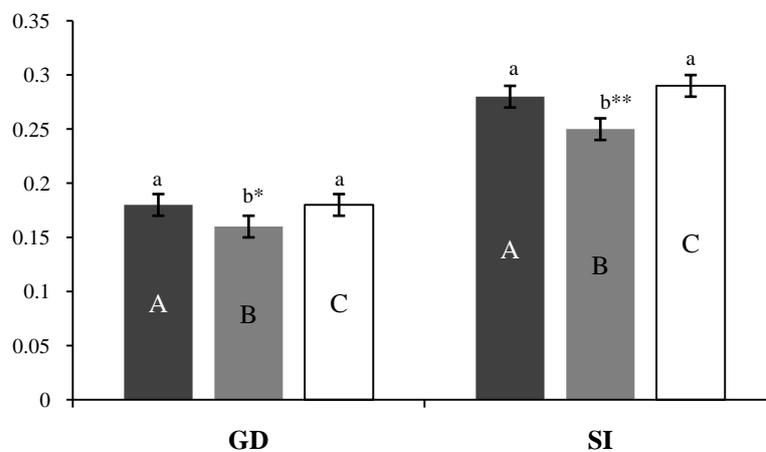
Increment and Eigenvalues: Axis 1 (11.3%, 4.0), Axis 2 (6.5%, 2.3). Legend shows markers with populations from fragmentation region A (black triangles), B (grey triangles) and C (empty triangles).



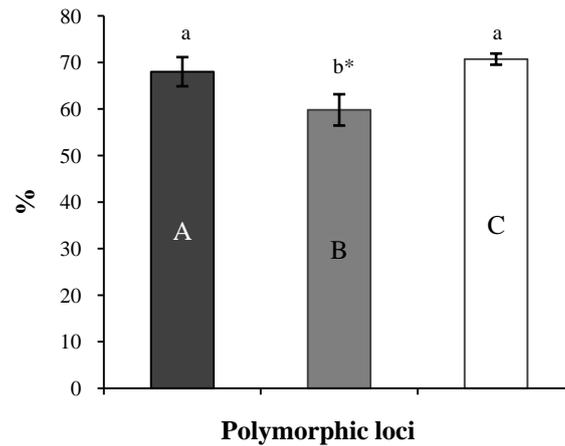
**Fig. 27. Spatial genetic structure of *Hemimeris racemosa*.**  
Mantel test between genetic ( $\Phi_{PTP}$ ) and geographical distances (GGD; in km) are positive correlated ( $r = 0.76$ ,  $p=0.001$ ).

#### *Impact of fragmentation degree on genetic structure*

At regional level, fragmentation region B had a significant lower genetic variation (GD, SI, PL) compared to region A and C (Fig. 28, Fig. 29). A low and similar genetic variation between populations ( $\Phi_{PT} = 0.05, 0.05, 0.04$ ) was present in each region (Table 16). Bayesian analysis revealed two groups as the most likely number of groupings for each fragmentation region (Table 17). Principal coordinates analysis of each fragmentation region showed no groupings and low values of explanation (Fig. 30, Fig. 31, Fig. 32). A Mantel test was not significant in fragmentation region A and B (Table 18) but revealed significant isolation by distance within fragmentation region C (Fig. 33).



**Fig. 28. Nei's gene diversity and Shannon's Index of *Hemimeris racemosa*.**  
Nei's gene diversity ( $GD \pm S.E.$ ) and Shannons Index ( $SI \pm S.E.$ ).  
Fragmentation regions (A, B, C) according to Table 11.



**Fig. 29. Percentage of polymorphic loci of *Hemimeris racemosa*.**

Percentage of polymorphic loci  $\pm$  S.E. Fragmentation region according to Table 11.

**Table 16. Analysis of molecular variance of *Hemimeris racemosa* (regional level).**

Based on 272 AFLP fragments. Proportion of genetic variation (%). Significance level ( $p < 0.001$ ) is based on 999 permutations.

| Fragmentation region with individuals and populations |     |    | Genetic variation   | Degrees of freedom | Sums of squares | Means squares | %   | Phi <sub>Pt</sub> |
|---|-----|----|---------------------|--------------------|-----------------|---------------|-----|-------------------|
| A   | 95  | 6  | Between populations | 5                  | 267.861         | 53.572        | 5%  | 0.05              |
|   |     |    | Within populations  | 89                 | 2687.254        | 30.194        | 95% |                   |
| B   | 102 | 7  | Between populations | 6                  | 275.041         | 45.840        | 5%  | 0.05              |
|   |     |    | Within populations  | 95                 | 2588.880        | 27.251        | 95% |                   |
| C   | 201 | 12 | Between populations | 11                 | 545.230         | 49.566        | 3%  | 0.04              |
|   |     |    | Within populations  | 189                | 5932.621        | 31.390        | 97% |                   |

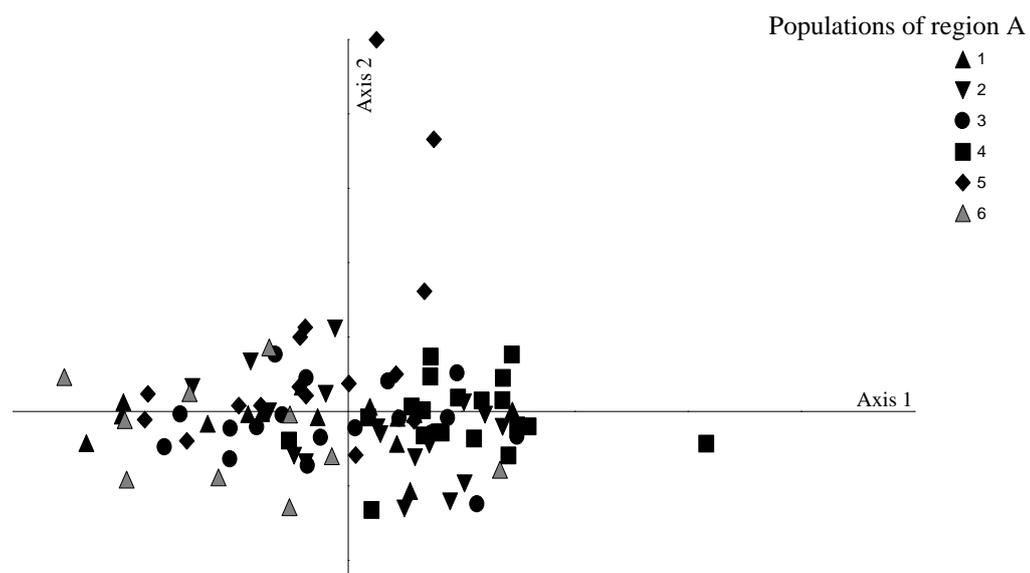
**Table 17. Bayesian model-based clustering likelihoods and model selection.**

10 replicate runs. Model selection for number of groups (k) present in the dataset. ‡Model selection method (Evanno *et al.* 2005)

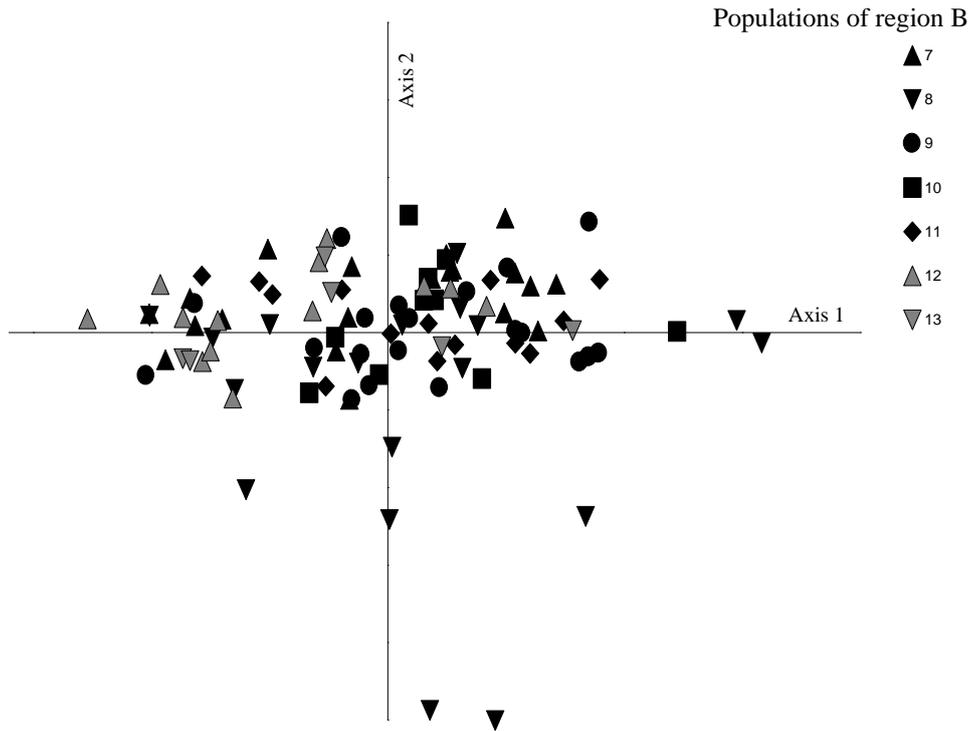
| Regions and runs (k) | Mean ln P(D) | S.E. ln P(D) | Variance ln P(D) | S.D. ln P(D) | $\Delta k$ ‡ |              |
|----------------------|--------------|--------------|------------------|--------------|--------------|--------------|
| A                    | 1            | -9533.8      | 0.22             | 0.48         | 0.69         |              |
|                      | 2            | -9319.38     | 2.21             | 48.97        | 7.00         | <b>25.32</b> |
|                      | 3            | -9282.16     | 2.36             | 55.53        | 7.45         | 17.95        |
|                      | 4            | -9378.69     | 47.71            | 22766.02     | 150.88       | 2.45         |
|                      | 5            | -9660.73     | 143.27           | 205261.15    | 453.06       | 1.10         |
|                      | 6            | -9582.14     | 138.05           | 190569.89    | 436.54       | 1.83         |
|                      | 7            | -9869.21     | 258.00           | 665648.49    | 815.87       | 1.04         |
|                      | 8            | -9613.53     | 46.36            | 21489.50     | 146.59       | 3.82         |

Table 17 cont.

|   |    |           |        |            |         |              |
|---|----|-----------|--------|------------|---------|--------------|
|   | 1  | -9337.87  | 0.37   | 1.36       | 1.17    |              |
|   | 2  | -9131.61  | 3.06   | 93.63      | 9.68    | <b>13.96</b> |
|   | 3  | -9060.42  | 5.35   | 286.24     | 16.92   | 12.71        |
|   | 4  | -9204.27  | 106.60 | 113625.14  | 337.08  | 3.40         |
| B | 5  | -10058.81 | 846.41 | 7164117.51 | 2676.59 | 0.78         |
|   | 6  | -9495.52  | 142.48 | 203008.19  | 450.56  | 3.65         |
|   | 7  | -9759.25  | 246.20 | 606135.15  | 778.55  | 1.26         |
|   | 8  | -10132.39 | 265.40 | 704389.46  | 839.28  | 1.19         |
|   | 9  | -10001.7  | 140.03 | 196075.80  | 442.80  | 1.32         |
|   | 1  | -20658.4  | 0.44   | 1.94       | 1.39    |              |
|   | 2  | -20389.82 | 1.92   | 36.70      | 6.06    | <b>37.99</b> |
|   | 3  | -20351.4  | 8.17   | 666.98     | 25.83   | 11.66        |
|   | 4  | -20614.2  | 106.85 | 114169.43  | 337.89  | 1.29         |
|   | 5  | -20728.16 | 139.47 | 194519.14  | 441.04  | 1.88         |
|   | 6  | -21149.94 | 144.66 | 209253.46  | 457.44  | 1.92         |
| C | 7  | -20865.24 | 94.33  | 88980.04   | 298.30  | 3.06         |
|   | 8  | -21381.59 | 138.87 | 192846.87  | 439.14  | 2.41         |
|   | 9  | -21594.44 | 201.10 | 404402.61  | 635.93  | 1.04         |
|   | 10 | -21520.09 | 134.66 | 181333.53  | 425.83  | 2.41         |
|   | 11 | -22336.06 | 221.08 | 488763.63  | 699.12  | 3.35         |
|   | 12 | -22229.67 | 588.25 | 3460438.90 | 1860.23 | 1.48         |
|   | 13 | -21818.02 | 211.62 | 447831.10  | 669.20  | 2.39         |
|   | 14 | -22416.91 | 243.64 | 593592.67  | 770.45  | 0.92         |



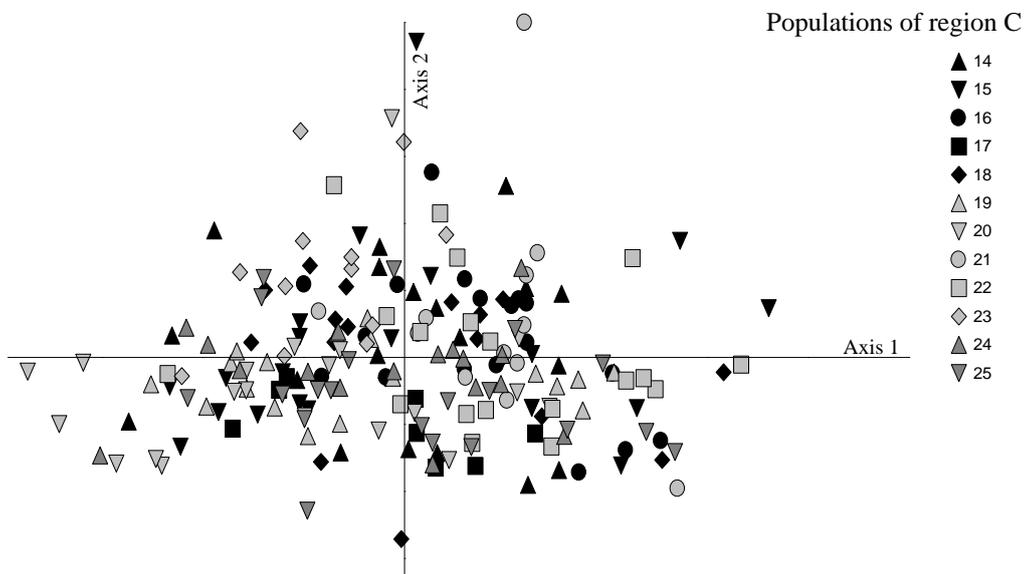
**Fig. 30. Principal coordinates analysis of *Hemimeris racemosa* in region A.**  
 Level of explanation and Eigenvalues of axis 1 (9.8%, 0.8) and axis 2 (6.2%, 0.5).  
 Legend shows populations according to Table 11.



**Fig. 31. Principal coordinates analysis of *Hemimeris racemosa* in regions B.**

Level of explanation and Eigenvalues of axis 1 (6.9%, 0.7) and axis 2 (9.6%, 0.5).

Legend shows populations according to Table 11.



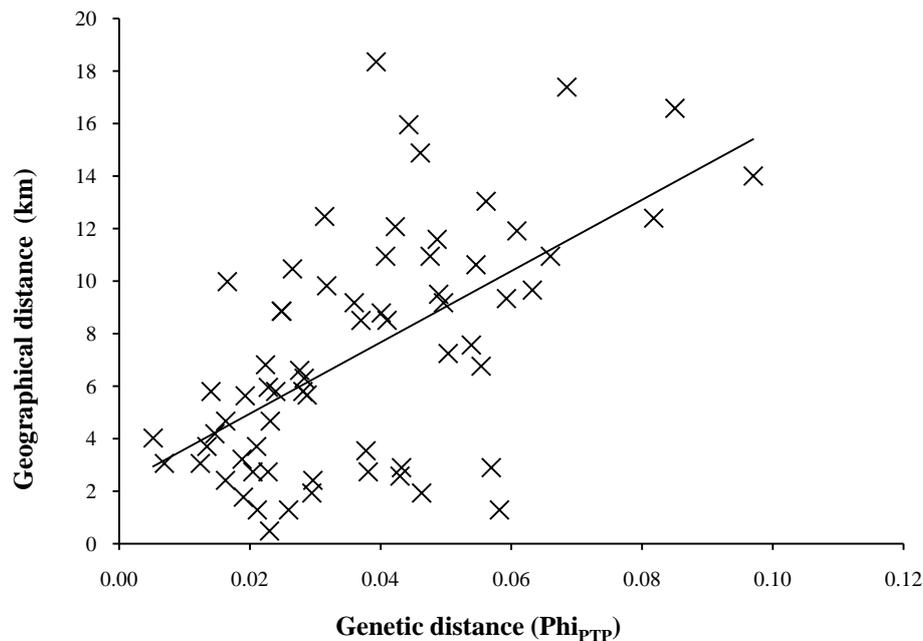
**Fig. 32. Principal coordinates analysis of *Hemimeris racemosa* in regions C.**

Level of explanation and Eigenvalues of axis 1 (4.1%, 0.9) and axis 2 (6.1%, 0.7).

Legend shows populations according to Table 11.

**Table 18. Mantel test with correlation coefficient of *Hemimeris racemosa*.**

| Fragmentation region | N   | r     | p            |
|----------------------|-----|-------|--------------|
| A                    | 95  | -0.36 | 0.15         |
| B                    | 102 | 0.55  | 0.08         |
| C                    | 201 | 0.58  | <b>0.001</b> |



**Fig. 33. Spatial genetic structure of *Hemimeris racemosa* in fragmentation region C.** Mantel test of genetic and geographical distances are positive correlated ( $r=0.6$ ,  $p=0.02$ ).

## Discussion

*Hemimeris racemosa* is a common annual plant in remnants of renosterveld vegetation. In order to characterize possible fragmentation effects on population genetics, 398 individuals from 25 remnants were collected. The accuracy of AFLP analysis increases with the number of loci (Travis *et al.* 1996) and in *H. racemosa* a large number of polymorphic DNA fragments (272 loci) have been effectively detected. Specifically, it was asked if low genetic variation within and high genetic variation between populations has been visible. Furthermore, a gradual genetic variation between regions was suspected, depending on fragment size and isolation. Different to expectations, results have indicated average genetic variation within and between populations and fragmentation regions of *H. racemosa*. No significant correlation between genetic variation and fragment size nor distance to neighbour fragment occurred.

*General genetic structure*

Comparing population genetic studies across species and with varying genetic markers is a delicate business. Nevertheless, the amount of genetic variation within populations of *H. racemosa* (Nei's gene diversity:  $GD=0.18$ , Shannon's Information index:  $SI=0.28$ , and percentage of polymorphic loci:  $PL=67\%$ ) was similar to allozyme data of outcrossing and annual species with  $H_{es}=0.19$  and  $P_s=59\%$ , (Hamrick and Godt 1996), RAPD data for annual, outcrossing, gravity-dispersed and early succession species with  $H_{pop}=0.13, 0.26, 0.21, 0.17$ , respectively (Nybom and Bartish 2000), and AFLP-data for *Eryngium alpinum* with  $SI=0.28$  for (Gaudeul *et al.* 2000). Genetic variation within populations is still high, indicating that genetic drift and inbreeding have not lowered genetic diversity in generation cycles during fragmentation. Little impact of fragmentation on genetic variation within populations was detected and loss of genetic variation that could potentially lead to reduced fitness seems not to be of significance. The genetic variation between populations of *H. racemosa* was low ( $\Phi_{pi}=0.09$ ) and similar to studies that have focussed on population distances smaller than 20 km, such as 0.07 for *Carex davalliana* (Hooftman *et al.* 2004), 0.06 for *Anthyllis vulneraria* (Honnay *et al.* 2006), and 0.05 for *Vincetoxicum hirundinaria* (Leimu and Mutikainen 2005). However, results are considerably lower than previously reported for other species, for example 0.13 for *Swertia perennis* (Lienert *et al.* 2002), 0.15 for *Primula veris* (Van Rossum *et al.* 2004), 0.17 for *Rutidosia leptorrhynchoides* (Young *et al.* 1999), 0.17 for *Primula vulgaris* (Van Rossum *et al.* 2004), 0.19 for *Succisa pratensis* (Hooftman *et al.* 2004), 0.44 for *Globularia bisnagarica* (Honnay *et al.* 2007), and 0.27 - 0.84 for *Pedicularis palustris* (Schmidt and Jensen 2000), as well as for meta-studies on allozyme data of outcrossing and animal pollinated species ( $G_{ST}=0.20$ , Hamrick and Godt 1996) and RAPD data of outcrossing species ( $\Phi_{st}=0.28$ , Nybom and Bartish 2000).  $G_{st}$  values greater than 0.1 are considered to show high genetic variation between populations and moderate genetic differentiation (Ellstrand and Elam 1993). Hence, little impact of fragmentation on genetic variation between populations of *H. racemosa* was visible. This point towards minor differentiation processes in *H. racemosa* and a low level of genetic drift. It might reflect the imprint of historical genetic exchange rather than recent gene flow (Templeton 1998). However, this can be precluded because of the long time between fragmentation and annual generation cycles. Bayesian clustering and PcoAs detected a spatial structure of two groups, separating the Botteleray region from the Tygerberg and Swartland region, which is contrary to predictions. This can be due to isolation by distance effects (Sork *et al.* 1999) and indicates a sufficient gene flow,

supported by a significant Mantel test and correlation between genetic and geographic distances for the entire dataset.

#### *Impact of fragmentation degree on genetic structure*

A significant lower genetic variation within populations was revealed in the Botteleray region (Nei's gene diversity  $GD=0.16$ , Shannon's index  $SI=0.25$ , and percentage of polymorphic loci  $PL=60\%$ ) compared to Tygerberg and Swartland region ( $GD=0.18$ ,  $SI=0.29$ , and  $PL\sim 69\%$ ). Hence, no reduction in genetic variation occurred in the highly fragmented Swartland region. Minor impact of fragmentation on genetic variation and little genetic drift can be assumed.

Very few studies with similar complex fragmentation setting exist so far. Previous studies have mainly examined population size assuming similar degree of isolation for all populations. This is the first attempt to study three different fragmentation settings based on anthropogenic habitat transformation. Minor differences in genetic variation between populations of *H. racemosa* ( $\Phi_{Pt}=0.04-0.05$ ) were identified between fragmentation regions. Similar values and sufficient gene flow were reported for *Hypochoeris radicata* ( $F_{ST}=0.04$ , Mix *et al.* 2006) within a small- and large-scale fragmentation setting. Minor fragmentation effects ( $F_{ST}=0.02$ ) for isolated local populations were reported for *Acer saccharum* (Foré *et al.* 1992, Young *et al.* 1993) where enhanced wind impact and dispersal has reduced genetic differentiation. In contrast, population differentiation was visible in long-lived, perennial *Swertia perennis* ( $F_{ST}=0.13$ , Lienert *et al.* 2002), occurring at one hectare mainland and small isolated remnants (0.5 ha, 1km distance).

The genetic constitution of *H. racemosa* populations indicates that fragmentation region (i.e. fragmentation degree) is of minor influence regarding inbreeding or genetic drift. Bayesian clustering detected a spatial structure of two groups in each fragmentation region Tygerberg and Swartland region. However, this is not reflected by PcoAs where populations have showed no distinct grouping. A significant Mantel test and correlation between genetic and geographic distances were only visible for Swartland and is due to isolation by distance effects (Sork *et al.* 1999). For Tygerberg and Botteleray, the idea of non-existing gene flow is rejected because gene flow was already visible in small and isolated Swartland fragments giving no reason why gene flow processes should not be present at the larger and less isolated fragments of the Tygerberg and Botteleray region.

Habitat fragmentation can negatively influence populations and gene flow amongst them (Young *et al.* 1996). These alterations may depend on landscape scale (Hutchison and Templeton 1999) and can be species and site-specific (Jacquemyn *et al.* 2003). Annual

species, such as *H. racemosa*, should retain much of their genetic variation among populations (Nybom and Bartish 2000), instead genetic variation was conserved within populations, which is more typical for long-lived species. The observed pattern of high genetic variation within populations and low genetic variation between them, further supports that fragmentation *per se* does not imply genetic isolation (Young *et al.* 1996). For *H. racemosa* it seems to be likely that one pollen or seed per generation is able to bridge populations, which is necessary to ensure sufficient gene flow and avoiding population differentiation (Slatkin 1985). Although *H. racemosa* is specialized regarding pollinators and collapse of pollination webs are reported for urban remnants (Pauw 2007), it seems that pollinators are still able to move between populations within an agricultural matrix. The second vector for gene flow is inter-population diaspore dispersal. Such dispersal is documented in fragmented European grassland species (Honnay *et al.* 2006) where livestock migrated between fragments and long-distance dispersal occurred (Mix *et al.* 2006). *H. racemosa* has small fruits without attachments for anemochorous or exozoochorous dispersal, but endozoochorous dispersal by migrating small game or livestock could ensure sufficient gene flow.

On the other hand, time span could have been too short to measure fragmentation effects on population genetic structure. However, this can be excluded for annual *H. racemosa* because fragmentation history dates back over a century and differentiation is already detectable after few generations (Epperson 1990). In the case of restricted recent gene flow, observed pattern would be an imprint of historic conditions with high gene flow. This situation would be supported by a regularly activated (e.g. mole rate, porcupine) and genetically diverse soil seed bank that could store much genetic variation (McCue and Holtsford 1998, Muir *et al.* 2004) and might buffer against genetic drift and differentiation (Honnay *et al.* 2008).

Nevertheless, data suggest that little impact of fragmentation on genetic variation within and between *H. racemosa* populations and regions is visible yet and studied populations are a panmictic meta-population with random and erratic gene flow and no barrier for pollination and dispersal.

#### *Implications for conservation*

Small plant populations are susceptible to extinction, due to loss of genetic variation via genetic drift, increased selfing and mating among related individuals. Even common species can be affected by population genetic consequences of habitat fragmentation (Honnay and Jacquemyn 2007). However, a lesson learnt from tropical forest fragments is that genetic

theory of small populations does not apply and ecological degradation is more important than genetic degradation at least for well dispersed species (Kramer *et al.* 2008). Results support the latter, that common species are more threatened by habitat loss than by genetic erosion. From a conservational point of view, Swartland fragments need special protection to avoid further habitat loss.

## **Conclusion**

The study of genetic variation in *Hemimeris racemosa* populations at the Cape lowlands has revealed minor fragmentation effects on population genetics, resulting in rejection of the proposed hypotheses. Specifically, considerable genetic variation within populations and minor genetic variation between populations still exist. Concerning fragmentation regions, no lower genetic variation within Swartland populations, a region suffering from severe fragmentation was found. Results suggest sufficient gene flow in the past; hence no effect of fragmentation is visible yet. Future research should incorporate genetic variation of the soil seed bank, as well as pollination and dispersal vectors, in order to estimate recent gene flow. Ongoing habitat loss might result in genetic erosion and needs to be avoided by the means of habitat protection, as well as the establishment of stepping stones and corridors. This is especially true for the small and isolated Swartland fragments. The landscape context and interesting setting of renosterveld fragments offers high potential for future population genetics studies on other species.

## **7 Does fragmentation really matter? Genetic variation within and between remnant populations of *Nemesia barbata* in the fragmented renosterveld of South Africa**

### **Abstract**

Fragmentation of plant populations is caused by anthropogenic land conversion and result in reduced population sizes and increasing population distances. In this process of habitat fragmentation, genetic variation within and between remnant plant populations can suffer. The species-rich, highly fragmented and endangered renosterveld vegetation of South Africa is a Mediterranean-type shrubland and once filled large proportions of the lowlands in the south-western Cape. After major agricultural land transformation, only ten percent of renosterveld are left in small isolated remnants. Hereby, geographical pattern and extent of land conversion resulted in three fragmentation situations with a varying degree of remnant size and isolation: large medium-distant, remnants at the Tygerberg area; semi-large, small-distant remnants at the Botteleray Hills; and small, large-distant remnants at the Swartland. In each of the regions, the annual herb *Nemesia barbata* was examined (seven, six and seven populations, respectively) and it was argued that fragmentation reduce general genetic variation and be affected by fragmentation degree. In contrast to this predictions, average genetic variation within populations (Nei's gene diversity=0.13) and high genetic variation between them ( $\Phi_{ST}$ =0.29) was encountered using AFLP analysis. Although fragmentation history dates back centuries, these values are similar to other studies on annual plant species with mixed breeding system. Furthermore, slight differences of genetic variation between populations ( $\Phi_{ST}$ ~0.26, ~0.31, ~0.21,) were detected when three fragmentation regions were analyzed separately. Lowest values were observed in the most severe fragmented region. It can be supposed that habitat fragmentation and its degree play a minor role in the genetic status of *Nemesia barbata* populations. It seems that historical and possibly recent gene flow may be the reason for the minor impact of habitat fragmentation.

## Introduction

Maintenance of genetic variation in fragmented populations is a major conservation goal in order to avoid inbreeding depressions (Saccheri *et al.* 1998), and to ensure adaptation (Hedrick and Miller 1992) and evolution of species (Lande and Barrowclough 1987). South African renosterveld vegetation is a species-rich, but highly fragmented and endangered shrubland, found in Mediterranean-type climate and on nutrient-rich soils (Rebelo *et al.* 2006). Here negative impacts on plant-pollinator mutualism were already observed, resulting in reduced genetic diversity within small conservation sites near urban areas (Pauw *et al.* 2004, Pauw 2007).

Habitat fragmentation (*sensu* Harrison and Bruna 1999) lead to habitat loss, reduced habitat size (Luijten *et al.* 2000), and smaller population sizes (Oostermeijer *et al.* 1996, Bruna and Kress 2002, Van Rossum *et al.* 2004). Furthermore, it will increase separation, isolation and interior-to-edge ratio of populations (Franklin *et al.* 2002). These factors can negatively affect biota in many regards (Kruess and Tscharntke 1994, Turner 1996, Amler *et al.* 1999). Eventually, it may result in reduced gene flow (Ouborg *et al.* 2006), loss of genetic variation (Fahrig 2001, Honnay *et al.* 2006), inbreeding and drift (Young *et al.* 1996), and the decrease of short- and long-term population viability (Ellstrand and Elam 1993).

Both, ecological and genetical processes can influence the genetic variation of species and populations (Linhart *et al.* 1981, Li and Adams 1989, Schmitt and Gamble 1990), which underpins their short-term fitness (Huenneke *et al.* 1991) and long-term persistence (Lee *et al.* 2006). Genetic variation strongly depends on plant functional traits and short-lived, non-woody, self-compatible, and early-successional species have a higher genetic variation between than within populations (Hamrick and Godt 1996, Nybom and Bartish 2000). Species rarity is another parameter and in general, less genetic variation occurs in rare species (Hamrick and Godt 1989, Gitzendanner and Soltis 2000, Cole 2003). Habitat fragmentation induced reduction of population size can be responsible for the decrease of genetic variation (Oostermeijer *et al.* 2003). This relation is mostly positive and more stronger in self-incompatible than self-compatible plants (Leimu *et al.* 2006). Montalvo *et al.* (1997) report that small populations are more affected by genetic drift than larger ones.

However, it is necessary to acknowledge population distance as a parameter in order to evaluate the spatial genetic structure (Ouborg *et al.* 2006). Here, neutral (Foré *et al.* 1992, Young *et al.* 1993) and negative effects (Schmidt and Jensen 2000, Lienert *et al.* 2002) on genetic variation are reported.

Gene flow by means of pollen and diaspores, ensures adaptation and evolution (Bishop 1972, Heywood 1991). However, habitat fragmentation can result in reduced gene flow and genetic variation (Young *et al.* 1996, Landergott *et al.* 2001). Naturally fragmented plant populations well observed (Larson *et al.* 1984, Ellstrand and Marshall 1985) and are relatively prone to inbreeding depression (Huenneke *et al.* 1991). However, anthropogenic induced fragmentation is far less studied (Lacy 1987, Lande and Barrowclough 1987, Robinson and Quinn 1992, Montalvo *et al.* 1997).

Renosterveld filled large proportions of the south-western Cape lowlands (Kemper *et al.* 1999). However, mainly agricultural land-transformation destroyed ninety percent of the former extent (von Hase *et al.* 2003a). The extent of conversion coupled with geomorphologic pattern (i.e. hills, hill ridge, lowland) resulted in three conspicuously fragmentation regions: large remnants with medium-distant at the Tygerberg area (region A), semi-large remnants with small-distant at the Botteleray Hills (region B), and small remnants with large-distant to neighbour at the Swartland (region C). Such situation is known as space-for-time substitution (Pickett 1989, Hargrove and Pickering 1992, Travis and Hester 2005, Honnay *et al.* 2009) enabling to compare less fragmented region A and B with region C, the hypothetical result of further habitat loss and isolation. *Nemesia barbata* (Scrophulariaceae) is a mixed breeder and annual herb, occurring in low abundances in all mentioned regions. It was hypothesized to find strong fragmentation effects on genetic variation. Concerning general genetic structure, a low genetic variation within and high genetic variation between populations was proposed. Populations in the three fragmentation regions should inherit gradual genetic variation, depending on fragment size and isolation.

## Material and Methods

### *Species description*

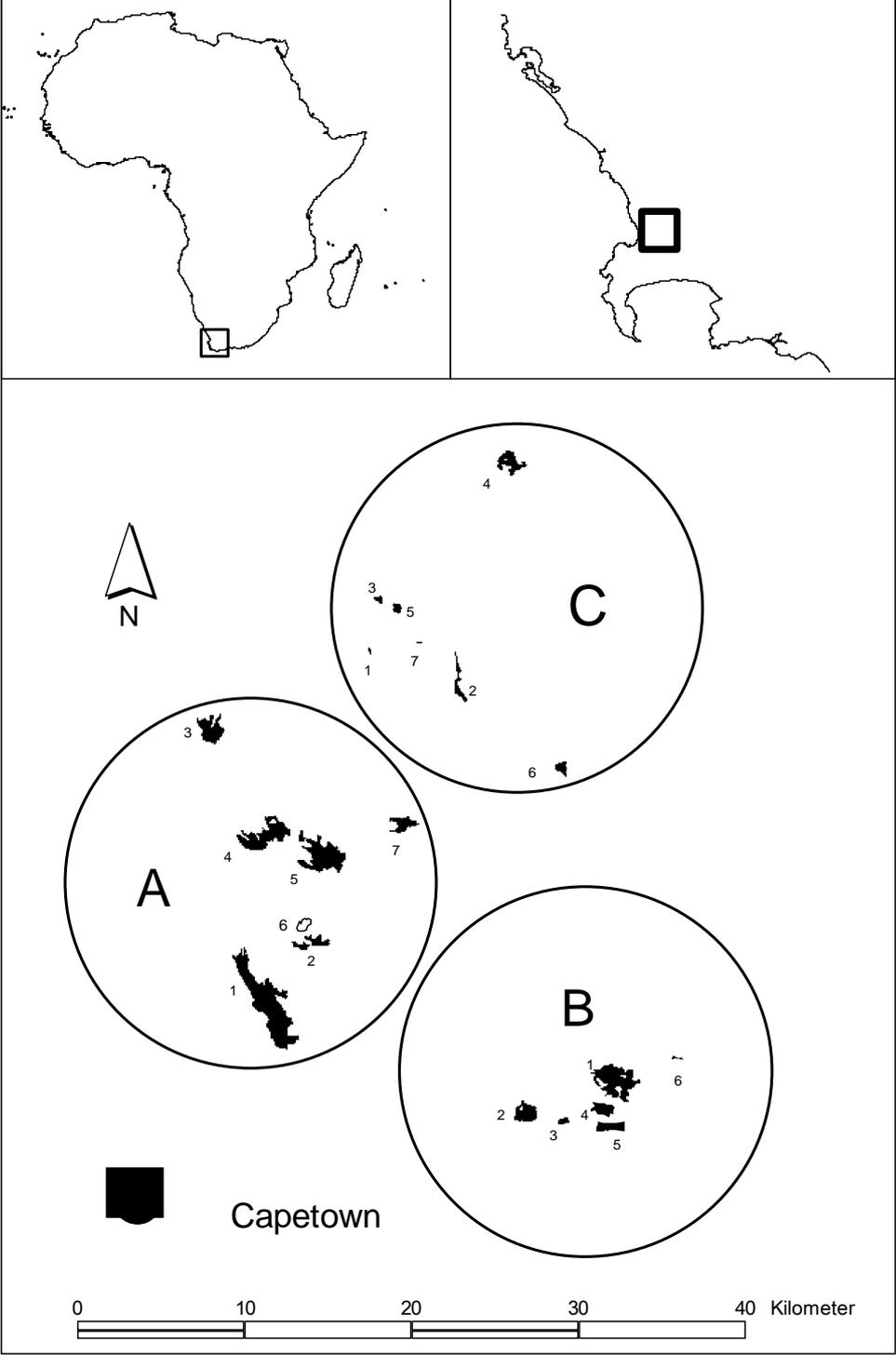
*Nemesia barbata* (Thunb.) Benth. (Hemimerideae, Scrophulariaceae, Olmstead *et al.* 2001) occurs in low abundances on sandy flats and slopes in the western CFR (Goldblatt and Manning 2000). This annual herb is diploid and has a chromosome number of  $x=18$  (Steiner 1996). *N. barbata* flowers from August to October, distinguished by white upper lip and blue lower lip and a short single spur (Fig. 34). The plant grows up to 30 cm in height and has opposite, ovate and toothed leaves (Goldblatt and Manning 2000). If hand-pollinated, *N. barbata* is self-compatible (Datson *et al.* 2006) and seeds are small and gravity-dispersed.



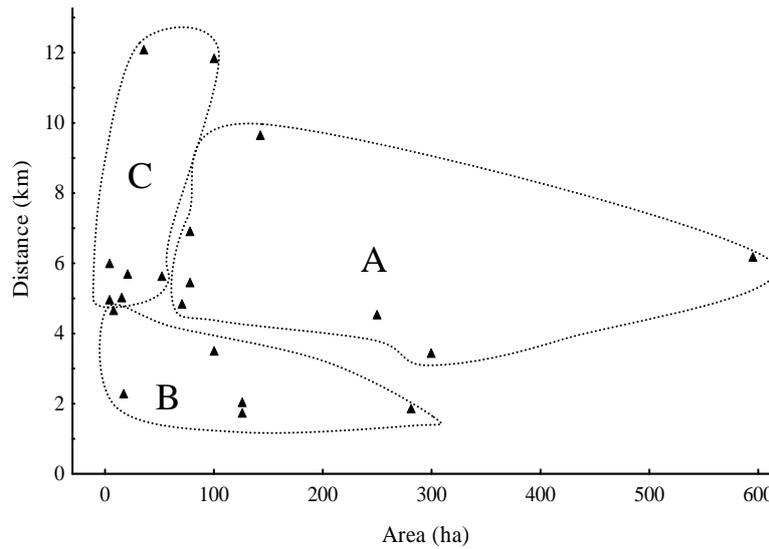
**Fig. 34. Habitus of *Nemesia barbata***  
Source: <http://www.livingfynbos.com>

#### *Sampling procedure*

The study area spans up to 40 km north and east of Cape Town in the Cape lowlands (Fig. 35). The sampling was performed in the three fragmentation regions (Fig. 35, Fig. 36) of West Coast Centre renosterveld (Rebello *et al.* 2006) covering virtually all available fragments within an 11 km radius. Genetic variation of 20 *N. barbata* populations (Table 19) was examined and it was estimated that fragment size equals population size. Mean distance to neighbour for each of the fragments was calculated (ArcView3.2, ESRI 2000) as an index resulting from the mean value of each edge-to-edge distance from one particular fragment to all other fragments. This index was made available for the entire dataset and done separately for each fragmentation region.



**Fig. 35. Sampling sites in different fragmentation regions of renosterveld.** Circles (diameter 22 km) and letters indicate fragmentation regions with sampling sites. For details see Table 19.



**Fig. 36. Sampling sites with fragment size and mean distance to neighbour fragment.** Fragmentation regions A, B and C are encircled and indicated by different letters. For details see Table 19.

**Table 19. Sampled populations of *Nemesia barbata*.**

Bold numbers are mean values of each category. † von Hase *et al.* (2003a) and estimations. \* Aerial photographs.

| Fragmentation region and population | Longitude (E) | Latitude (S) | Fragment size (ha) † | Mean distance to neighboring remnants (km) ± S.E |
|-------------------------------------|---------------|--------------|----------------------|--|
| 1 Tygerberg                         | 18°35'39"     | 33°52'37"    | 595                  | 6.22 ± 1.60                                      |
| 2 Kanonkop                          | 18°36'16"     | 33°49'35"    | 78                   | 5.47 ± 1.62                                      |
| 3 Koeberg                           | 18°33'28"     | 33°42'49"    | 141                  | 9.71 ± 1.16                                      |
| A 4 Porquepine                      | 18°35'15"     | 33°46'10"    | 248                  | 4.59 ± 0.78                                      |
| 5 Meerendal                         | 18°37'23"     | 33°46'59"    | 298                  | 3.49 ± 0.80                                      |
| 6 Kanonkop Slope*                   | 18°36'08"     | 33°49'05"    | 70                   | 4.85 ± 1.46                                      |
| 7 Sondagsfontein                    | 18°39'44"     | 33°45'50"    | 78                   | 6.97 ± 1.09                                      |
|                                     |               |              | <b>215.43 ± 71.8</b> | <b>5.90 ± 0.77</b>                               |
| 1 Koop PNR                          | 18°45'55"     | 33°54'14"    | 281                  | 1.87 ± 0.46                                      |
| 2 Zevenwacht                        | 18°43'35"     | 33°55'16"    | 100                  | 3.54 ± 0.91                                      |
| B 3 Mooiplaas                       | 18°44'32"     | 33°55'29"    | 17                   | 2.32 ± 0.70                                      |
| 4 Wolf bott*                        | 18°45'58"     | 33°54'53"    | 125                  | 1.77 ± 0.59                                      |
| 5 Wolf top*                         | 18°46'15"     | 33°55'17"    | 125                  | 2.09 ± 0.61                                      |
| 6 Koop East*                        | 18°46'58"     | 33°54'04"    | 7                    | 4.70 ± 0.78                                      |
|                                     |               |              | <b>109.17 ± 40.4</b> | <b>2.71 ± 0.48</b>                               |
| 1 Middlepos                         | 18°38'37"     | 33°40'14"    | 4                    | 6.04 ± 1.77                                      |
| 2 Klipheuwel                        | 18°41'23"     | 33°41'52"    | 52                   | 5.66 ± 1.32                                      |
| 3 Remshoogte S                      | 18°38'55"     | 33°38'33"    | 20                   | 5.71 ± 1.79                                      |
| C 4 Helderfontein                   | 18°42'52"     | 33°34'03"    | 100                  | 11.86 ± 1.11                                     |
| 5 Remshoogte L*                     | 18°39'29"     | 33°38'51"    | 14                   | 5.07 ± 1.74                                      |
| 6 Klapmuts                          | 18°44'45"     | 33°44'04"    | 34                   | 12.10 ± 1.14                                     |
| 7 Bonnie Doon                       | 18°40'13"     | 33°39'56"    | 3                    | 5.02 ± 1.59                                      |
|                                     |               |              | <b>32.43 ± 13.0</b>  | <b>7.35 ± 1.20</b>                               |

*DNA isolation, AFLP and statistical analysis*

DNA isolation, AFLP analysis and sequencer procedure are described in detail at chapter six, page 69-71. Selective amplifications were performed with the three primer combinations (MseI-CTA/EcoRI-ACC, MseI-CAC/EcoRI-AAG, MseI-CTC/EcoRI-ACA). The statistical analysis of the AFLP data matrix was similar as described in chapter six, page 71-72. However, for each predefined number of  $K$  (2-22) 10 iterations were run in the Bayesian analysis.

**Results***General genetic structure*

AFLP analyses revealed a total of 206 fragments: MseI-CTA/EcoRI-ACC (76 fragments), MseI-CAC/EcoRI-AAG (63 fragments), MseI-CTC/EcoRI-ACA (67 fragments). Mean number of polymorphic loci was  $40.0 \% \pm 2.6$  (Table 20). Mean Nei's Gene Diversity was  $0.13 \pm 0.01$ . Mean Shannon's Information Index was  $0.19 \pm 0.01$ . Highest genetic variation was found at population B-5 and C-2 (GD=0.07, SI=0.10) and C-2, PL=59.7 (%). Lowest genetic variation was found at population A-7 (GD=0.07, SI=0.10, PL=20.9 %) and C-7 (PL=20.9 %). No significant correlation of fragment size and distance with genetic variation was visible (Table 21). Analyses of molecular variances ( $\Phi_{Pt}=0.29$ ) show high genetic variation between populations (Table 22). Two groups are the most likely for the entire dataset (Table 23, Fig. 37, Fig. 38) with random individual allocation (Fig. 39). Principal coordinates analysis revealed a distinct grouping with one outlier group (Fig. 40). Mantel test show significant isolation by distance for the entire dataset (Fig. 41).

**Table 20. Genetic variation within populations of *Nemesia barbata*.**

Fragmentation region according to Table 19. Bold numbers show mean values of each fragmentation region. Sampling number (N).

| Region and population number | N  | Nei's gene diversity (GD $\pm$ S.E.) | Shannons Index (SI $\pm$ S.E.) | Percentage of polymorphic loci (PL) |
|------------------------------|----|--------------------------------------|--------------------------------|-------------------------------------|
| 1                            | 14 | 0.13 $\pm$ 0.01                      | 0.21 $\pm$ 0.02                | 50.5                                |
| 2                            | 7  | 0.12 $\pm$ 0.01                      | 0.18 $\pm$ 0.02                | 35.9                                |
| 3                            | 13 | 0.10 $\pm$ 0.01                      | 0.16 $\pm$ 0.02                | 34.6                                |
| A 4                          | 8  | 0.11 $\pm$ 0.01                      | 0.17 $\pm$ 0.02                | 30.1                                |
| 5                            | 12 | 0.14 $\pm$ 0.01                      | 0.22 $\pm$ 0.02                | 48.5                                |
| 6                            | 6  | 0.08 $\pm$ 0.01                      | 0.12 $\pm$ 0.02                | 24.3                                |
| 7                            | 5  | 0.07 $\pm$ 0.01                      | 0.10 $\pm$ 0.01                | 20.9                                |

Table 20 cont.

|                 |   |    |                    |                    |                    |
|-----------------|---|----|--------------------|--------------------|--------------------|
| B               | 1 | 18 | 0.13 ± 0.01        | 0.19 ± 0.02        | 44.2               |
|                 | 2 | 20 | 0.13 ± 0.01        | 0.20 ± 0.02        | 46.6               |
|                 | 3 | 20 | 0.14 ± 0.01        | 0.22 ± 0.02        | 48.5               |
|                 | 4 | 5  | 0.11 ± 0.01        | 0.16 ± 0.02        | 29.6               |
|                 | 5 | 6  | 0.17 ± 0.01        | 0.26 ± 0.02        | 49.0               |
|                 | 6 | 6  | 0.11 ± 0.01        | 0.17 ± 0.02        | 34.5               |
| C               | 1 | 20 | 0.15 ± 0.01        | 0.24 ± 0.02        | 53.4               |
|                 | 2 | 20 | 0.17 ± 0.01        | 0.26 ± 0.02        | 59.7               |
|                 | 3 | 20 | 0.16 ± 0.01        | 0.24 ± 0.02        | 51.5               |
|                 | 4 | 10 | 0.13 ± 0.01        | 0.21 ± 0.02        | 45.2               |
|                 | 5 | 7  | 0.16 ± 0.01        | 0.24 ± 0.02        | 44.7               |
|                 | 6 | 3  | 0.10 ± 0.01        | 0.15 ± 0.02        | 27.2               |
|                 | 7 | 2  | 0.09 ± 0.01        | 0.13 ± 0.02        | 20.9               |
| All populations |   |    | <b>0.13 ± 0.01</b> | <b>0.19 ± 0.01</b> | <b>40.0 ± 2.60</b> |

**Table 21. Pearson correlation coefficient of fragment size and distance with genetic variation.** Significant correlations are bold and indicated (\* P<0.05).

| Correlation of  | fragment size with |       |       | mean distance to neighboring remnants |        |        |
|-----------------|--------------------|-------|-------|---------------------------------------|--------|--------|
|                 | GD                 | SI    | PL    | GD                                    | SI     | PL     |
| All populations | 0.017              | 0.061 | 0.185 | -0.269                                | -0.211 | -0.178 |

**Table 22. Results of analysis of molecular variance of *Nemesia barbata*.**

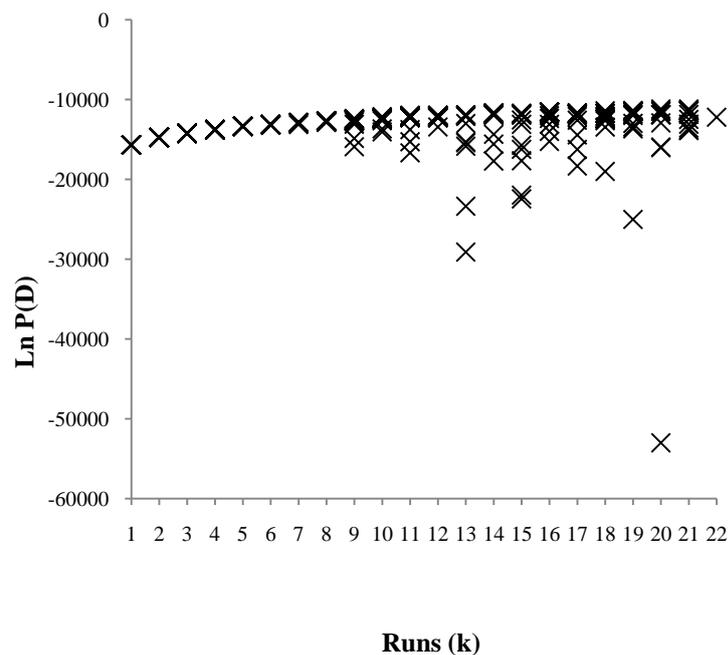
Based on 206 AFLP fragments. Proportion of genetic variation (%). Significance level ( $p > 0.001$ ) is based on 999 permutations.

| Individuals | Populations | Genetic variation   | Degrees of freedom | Sums of squares | Means squares | %   | Phi <sub>Pt</sub> |
|-------------|-------------|---------------------|--------------------|-----------------|---------------|-----|-------------------|
| 222         | 20          | Between populations | 19                 | 1653.111        | 87.006        | 29% | 0.29              |
|             |             | Within populations  | 202                | 3279.308        | 16.234        | 71% |                   |

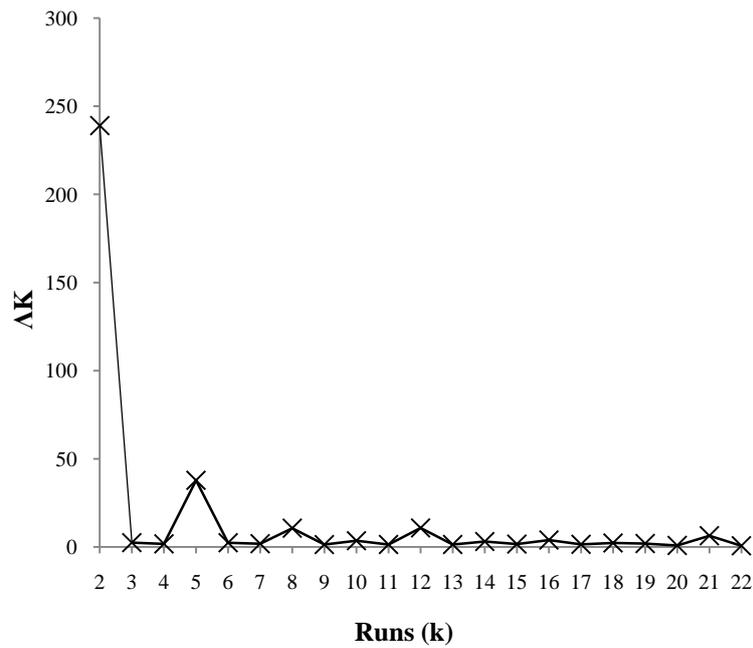
**Table 23. Bayesian model-based clustering likelihoods and model selection.**

True number of groups (k) and log probability of data Ln P(D). Ad hoc quantity based on rate of change of likelihood function with respect to K calculation ( $\Delta K$ ). 10 replicate runs. Values of K>10 are not reported due to low likelihood. Model selection for number of groups (k) present in the dataset. ‡ Model selection method (Evanno *et al.* 2005).

| k  | Mean ln P(D) | S.E. ln P(D) | Variance ln P(D) | S.D. ln P(D) | $\Delta k$ ‡  |
|----|--------------|--------------|------------------|--------------|---------------|
| 1  | -15668.83    | .23          | .54              | 0.74         |               |
| 2  | -14741.44    | .537         | 2.88             | 1.70         | <b>239.03</b> |
| 3  | -14219.88    | 11.62        | 1350.79          | 36.75        | 2.50          |
| 4  | -13765.22    | 12.11        | 1467.66          | 38.31        | 1.81          |
| 5  | -13353.04    | 1.67         | 27.81            | 5.27         | 37.86         |
| 6  | -13140.52    | 12.05        | 1451.31          | 38.10        | 2.42          |
| 7  | -12917.03    | 23.83        | 5679.03          | 75.36        | 1.92          |
| 8  | -12716.75    | 22.45        | 5039.13          | 70.99        | 10.66         |
| 9  | -13161.04    | 384.08       | 1475159.83       | 1214.56      | 1.42          |
| 10 | -12674.49    | 204.05       | 416350.51        | 645.25       | 3.59          |

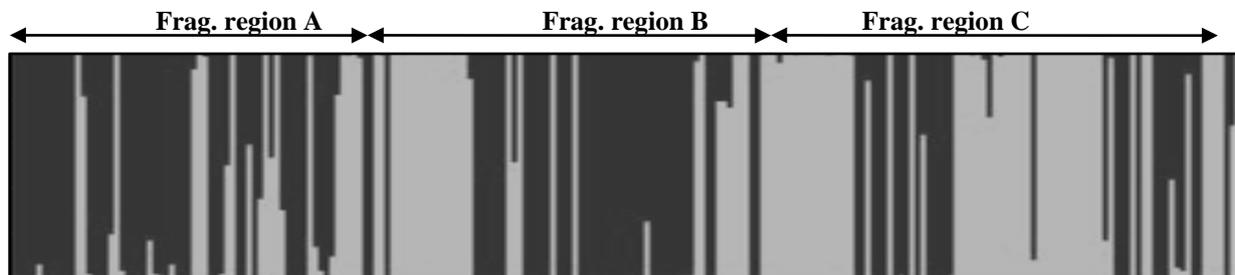
**Fig. 37. Bayesian analysis using logarithmic likelihood of group allocation.**

True number of groups (k) and log probability of data Ln P(D). Calculation based on 206 AFLP fragments for 222 *Nemesia barbata* individuals from 20 populations. 10 replicates revealed the most likely number of groups K=2 with lowest variance of Ln P is S.D.=1.7.

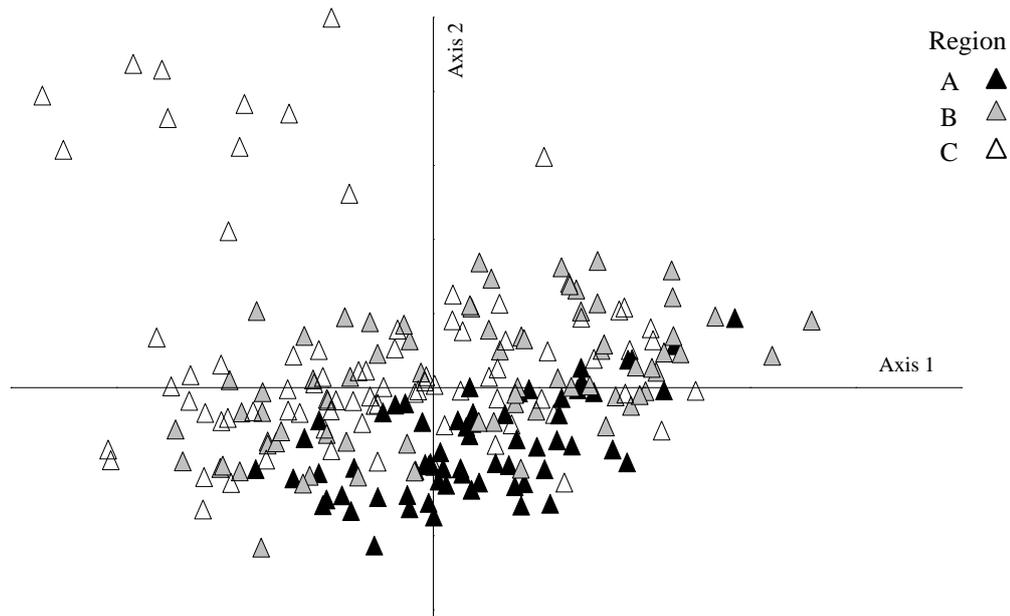


**Fig. 38. Bayesian analysis using logarithmic likelihood of group allocation.**

True number of groups (k). Ad hoc quantity based on rate of change of likelihood function with respect to K calculation ( $\Delta K$ ). Calculation based on 206 AFLP fragments for 222 *Nemesia barbata* individuals from 20 populations. 10 replicates revealed the most likely number of groups  $K=2$  with lowest variance and highest  $\Delta K=239.0$ .

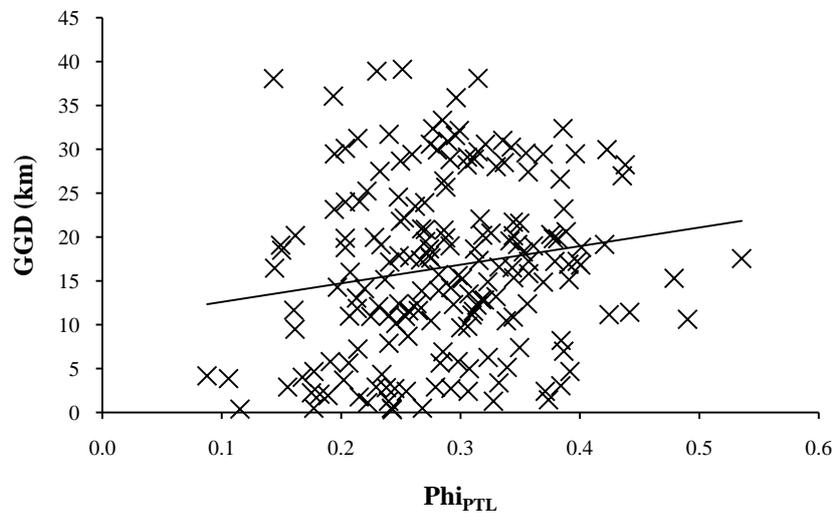


**Fig. 39. Individual assignments to  $K=2$  demes.**



**Fig. 40. Principal coordinates analysis of 222 *Nemesia barbata* individuals.**

Increment and Eigenvalues: Axis 1 (17.3%, 1.0), Axis 2 (12.0%, 0.7). The legend shows markers with populations from fragmentation region A (black markers), B (grey markers) and C (white markers).



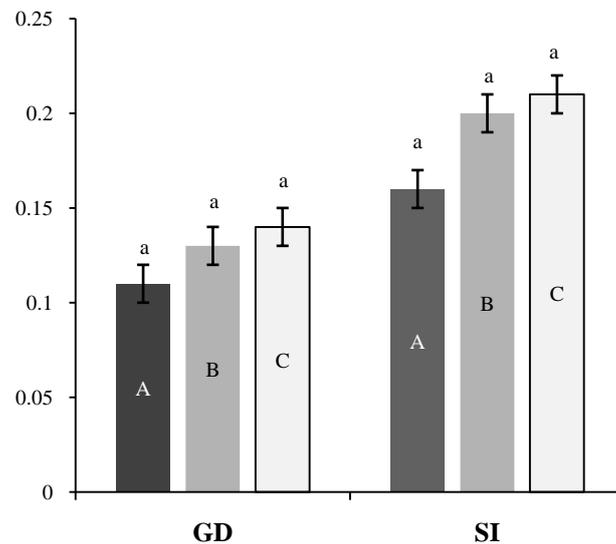
**Fig. 41. Spatial genetic structure of *Nemesia barbata*.**

Mantel test between genetic ( $\Phi_{iPTL}$ ) and geographical distances (GGD; km) positive correlated ( $r=0.166$ ,  $p=0.033$ ).

#### *Impact of fragmentation degree on genetic structure*

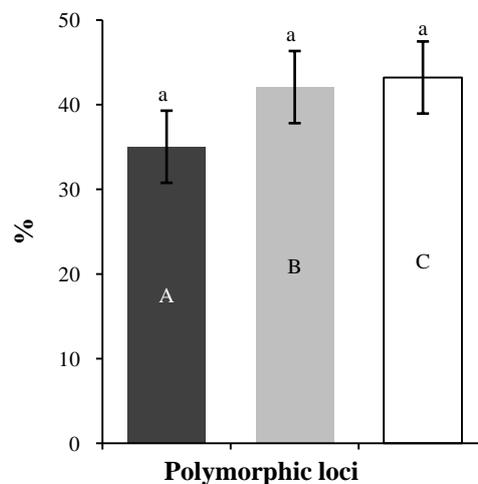
At regional level, no significant different genetic variation was found between fragmentation regions (Fig. 42, Fig. 43). If each fragmentation region is calculated separately, a very similar and high genetic variation between populations ( $\Phi_{Pi}=0.27$ ,  $0.31$ ,  $0.21$ ) is present (Table 24).

Two groups are the most likely number of groups for each fragmentation region (Bayesian analysis, Table 25). Although clusters were found in principal coordinates analysis of each fragmentation region, they show low values of explanation (Fig. 44, Fig. 45, Fig. 46). Significant isolation by distance was revealed in the Tygerberg region only (Mantel test, Table 26, Fig. 47).



**Fig. 42. Nei's Gene diversity and Shannon's Index of *Nemesia barbata*.**

Nei's gene diversity (GD ± S.E.) and Shannons Index (SI ± S.E.). Fragmentation region (A, B, C) according to Table 19. ANOVA ( $p < 0.05$ ) for group comparison of GD ( $F = 2.20$ ) and SI ( $F = 2.06$ ). Significant region comparison is depicted by different letters.



**Fig. 43. Percentage of polymorphic loci of *Nemesia barbata*.**

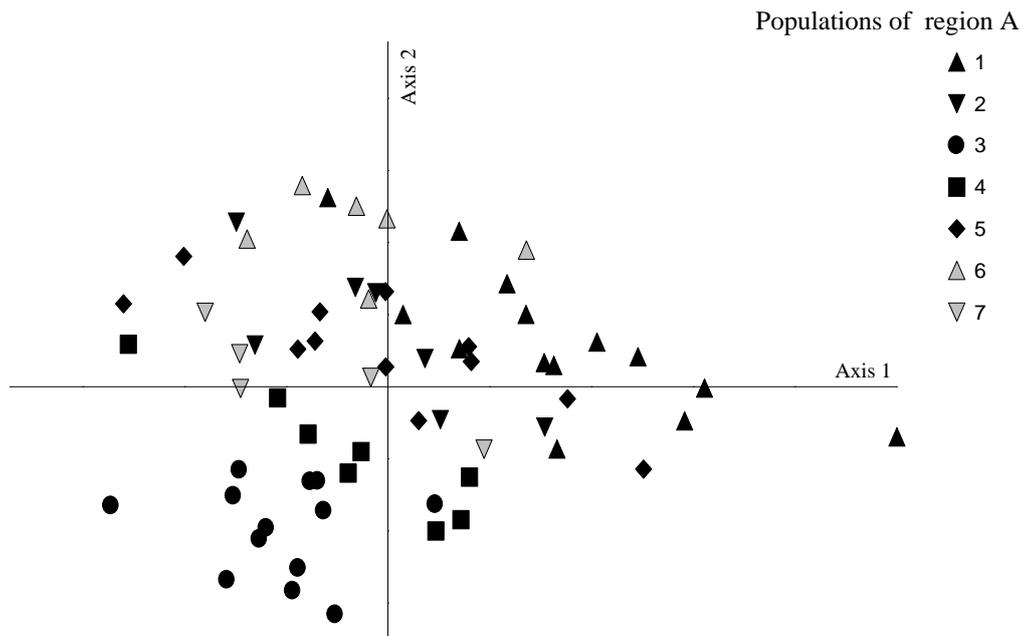
Percentage of polymorphic loci (PL ± S.E.). Fragmentation region according to Table 19. ANOVA ( $p < 0.05$ ) for group comparison of PL ( $F = 1.00$ ). Significant region comparison is depicted by different letters.

**Table 24. Results of analysis of molecular variance of *Nemesia barbata* (regional level).**Based on 206 AFLP fragments. Proportion of genetic variation (%). Significance level ( $p > 0.001$ ) based on 999 permutations.

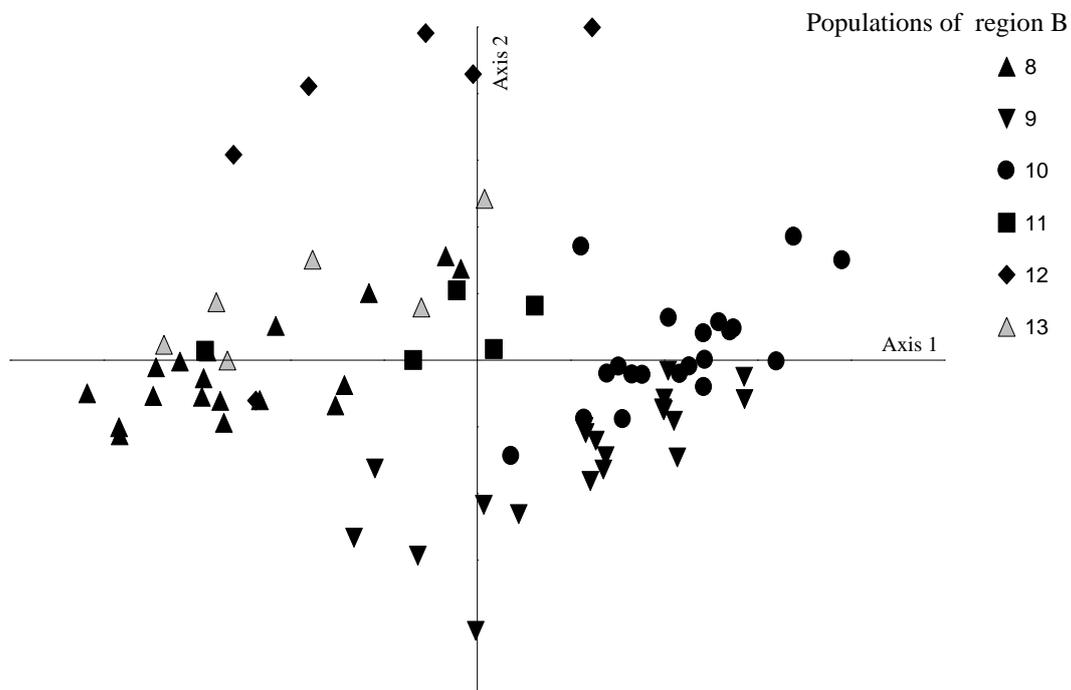
| Fragmentation region<br>with individuals and populations |    |   | Genetic variation   | Degrees<br>of freedom | Sums<br>of squares | Means<br>squares | %   | Phi <sub>Pt</sub> |
|--|----|---|---------------------|-----------------------|--------------------|------------------|-----|-------------------|
| A  | 65 | 7 | Between populations | 6                     | 363.385            | 60.564           | 26% | 0.27              |
|  |    |   | Within populations  | 58                    | 821.907            | 14.171           | 74% |                   |
| B  | 75 | 6 | Between populations | 5                     | 466.731            | 93.346           | 31% | 0.31              |
|  |    |   | Within populations  | 69                    | 1043.589           | 15.124           | 69% |                   |
| C  | 82 | 6 | Between populations | 6                     | 447.664            | 74.611           | 21% | 0.21              |
|  |    |   | Within populations  | 75                    | 1413.812           | 18.851           | 79% |                   |

**Table 25. Bayesian model-based clustering likelihoods and model selection.**10 replicate runs. Model selection for number of groups (k) present in the dataset. ‡Model selection method (Evanno *et al.* 2005).

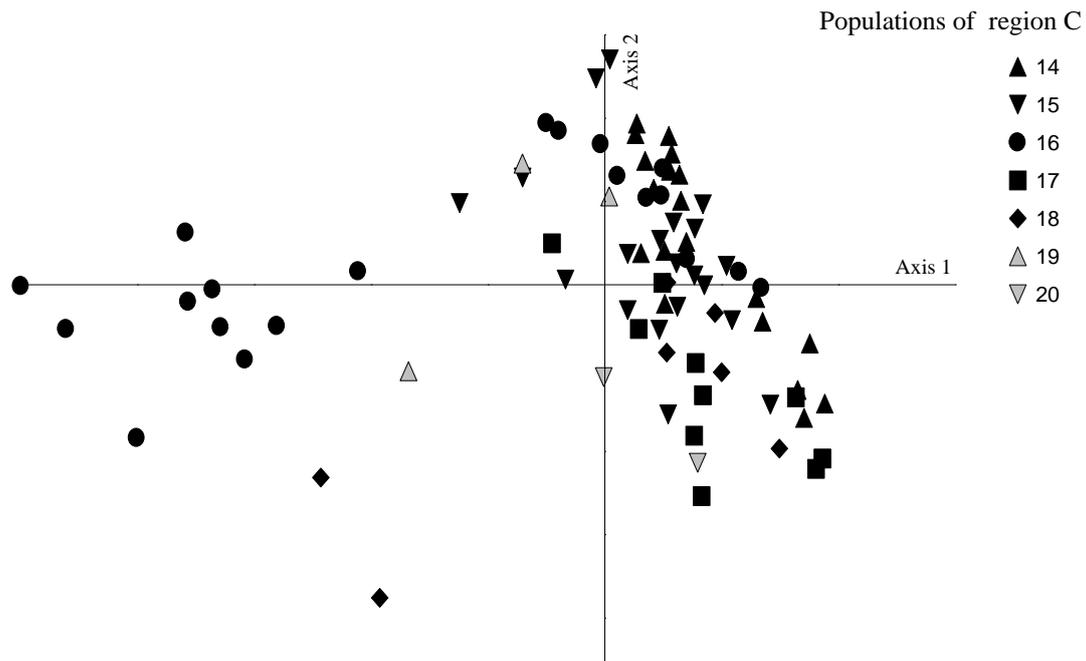
| Regions and runs (k) | Mean ln P(D) | S.E. ln P(D) | Variance ln P(D) | S.D. ln P(D) | Δk ‡    |               |
|----------------------|--------------|--------------|------------------|--------------|---------|---------------|
| A                    | 1            | -3840.70     | 0.42             | 1.79         | 1.34    |               |
|                      | 2            | -3609.51     | 6.36             | 403.88       | 20.10   | 7.84          |
|                      | 3            | -3530.04     | 121.71           | 148131.03    | 384.88  | 0.73          |
|                      | 4            | -3239.06     | 7.93             | 628.25       | 25.06   | 7.47          |
|                      | 5            | -3135.39     | 4.10             | 168.35       | 12.98   | <b>15.42</b>  |
|                      | 6            | -3231.79     | 74.40            | 55353.14     | 235.27  | 0.99          |
|                      | 7            | -3107.21     | 28.58            | 8169.68      | 90.39   | 1.82          |
|                      | 8            | -3146.99     | 29.79            | 8872.98      | 94.20   | 1.21          |
|                      | 9            | -3266.51     | 102.21           | 104476.07    | 323.23  | 0.71          |
| B                    | 1            | -4873.26     | 0.42             | 1.75         | 1.32    |               |
|                      | 2            | -4325.04     | 0.56             | 3.10         | 1.76    | <b>196.54</b> |
|                      | 3            | -4123.03     | 5.32             | 283.47       | 16.84   | 5.19          |
|                      | 4            | -3990.72     | 16.69            | 2785.33      | 52.78   | 2.86          |
|                      | 5            | -3876.74     | 36.38            | 13231.72     | 115.03  | 2.87          |
|                      | 6            | -3982.36     | 144.19           | 207911.03    | 455.97  | 1.80          |
|                      | 7            | -4190.11     | 349.03           | 1218232.87   | 1103.74 | 1.27          |
|                      | 8            | -4228.27     | 333.92           | 1115020.93   | 1055.95 | 0.80          |
| C                    | 1            | -5940.31     | 0.41             | 1.70         | 1.30    |               |
|                      | 2            | -5489.63     | 14.61            | 2135.00      | 46.21   | 3.86          |
|                      | 3            | -5196.16     | 12.68            | 1608.08      | 40.10   | 2.00          |
|                      | 4            | -4944.33     | 14.39            | 2071.65      | 45.52   | 3.69          |
|                      | 5            | -4829.30     | 54.68            | 29898.94     | 172.91  | 1.45          |
|                      | 6            | -4671.90     | 18.21            | 3317.66      | 57.60   | <b>6.38</b>   |
|                      | 7            | -4806.80     | 151.61           | 229847.86    | 479.42  | 1.21          |
|                      | 8            | -4760.59     | 153.13           | 234479.61    | 484.23  | 2.37          |
|                      | 9            | -5157.17     | 310.78           | 965839.56    | 982.77  | 0.83          |



**Fig. 44. Principal coordinates analysis of *Nemesia barbata* in regions A.** Level of explanation and Eigenvalues of axis 1 (19.8%, 0.2) and axis 2 (19.2%, 0.2). Legend shows populations according to Table 20.



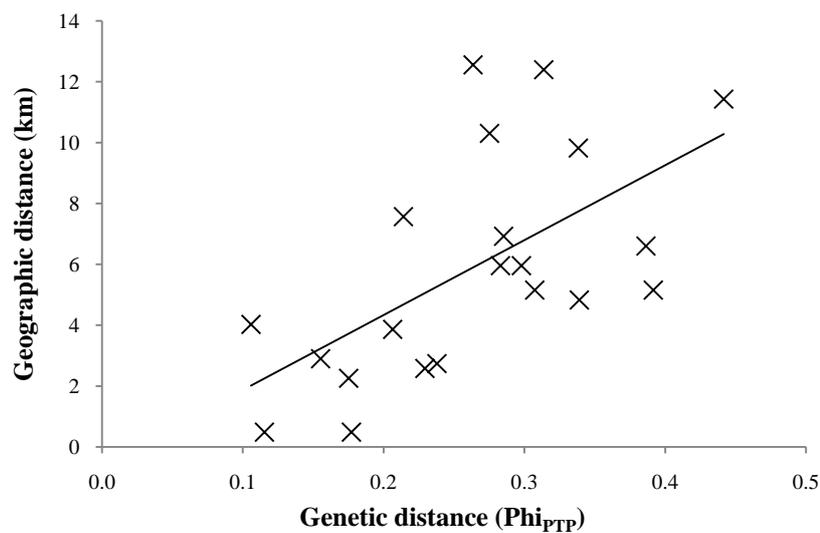
**Fig. 45. Principal coordinates analysis of *Nemesia barbata* in regions B** Level of explanation and Eigenvalues of axis 1 (29.2%, 0.5) and axis 2 (15.8%, 0.3). Legend shows populations according to Table 20.



**Fig. 46. Principal coordinates analysis of *Nemesia barbata* in regions C.** Level of explanation and Eigenvalues of axis 1 (22.8 %, 0.6) and axis 2 (15.5 %, 0.4). Legend shows populations according to Table 20.

**Table 26. Mantel test results of *Nemesia barbata* in three fragmentation regions.**

| Fragmentation region | N  | r    | p            |
|----------------------|----|------|--------------|
| A                    | 65 | 0.61 | <b>0.004</b> |
| B                    | 75 | 0.20 | 0.265        |
| C                    | 82 | 0.17 | 0.355        |



**Fig. 47. Spatial genetic structure of *Nemesia barbata* in fragmentation region A.** Mantel test of genetic ( $\Phi_{PTP}$ ) and geographical distances (km) are positive correlated ( $r=0.61$ ,  $p=0.004$ ).

## Discussion

*Nemesia barbata* is a common annual, mixed breeding herb occurring in low abundances in remnants of renosterveld vegetation. In this study 222 individuals from 20 remnants were collected to characterize possible fragmentation effects on population genetics of the species. Low genetic variation within populations and high genetic variation between them, as well as gradual genetic variation between regions was suspected. However, results revealed considerable genetic variation within and between populations and fragmentation regions. No significant correlation between genetic variation and fragment size nor distance to neighbour fragment was visible.

### *General genetic structure*

Genetic variation within populations of *N. barbata* ( $GD=0.13$ ,  $SI=0.19$ ,  $PL=40\%$ ) was similar to annual, mixed breeding species with  $H_{es}=0.12$ ,  $P_s=40.3\%$  (allozyme data, Hamrick and Godt 1996), and annual species with  $H_{pop}=0.13$  (RAPD data, Nybom and Bartish 2000). The remarkable high genetic variation has not lowered genetic diversity in generation cycles during fragmentation indicating little effect of genetic drift and inbreeding.

Genetic variation between populations appeared to be high ( $\Phi_{iP_t}=0.29$ ) and consistent for gravity-dispersed and mixed breeding species with  $G_{ST}=0.25$  (allozyme data, Hamrick and Godt 1996), and mixed breeding species with  $\Phi_{iST}=0.27$  (RAPD data, Nybom and Bartish 2000). Substantially higher  $\Phi_{iP_t}$  were found in studies on other herbaceous plants that focussed on population distances smaller than 20 km, for example, *Vincetoxicum hirundinaria* (0.05, Leimu and Mutikainen 2005), *Primula veris* (0.06, Van Rossum *et al.* 2004), *Carex davalliana* (0.07, Hooftman *et al.* 2004), *Swertia perennis* (0.13, Lienert *et al.* 2002), *Primula vulgaris* (0.17, Van Rossum *et al.* 2004), *Rutidosia leptorrhynchoides* (0.19, Young *et al.* 1999).

Two spatial groups without a clear individual assignment to a particular region were detected in the Bayesian clustering. This was mirrored by one large cluster in PcoA and an outlier group, possibly responsible for the detection of two groups in the Bayesian analysis. Isolation by distance effects (Sork *et al.* 1999) and sufficient gene flow were accounted in a significant correlation between genetic and geographic distances.

### *Impact of fragmentation degree on genetic structure*

Genetic variation within populations ( $GD$ ,  $SI$ ,  $PL$ ) was not significantly different between Tygerberg, Botteleray and Swartland region. Because no reduction in genetic variation in the

highly fragmented Swartland region was observed, minor impact of fragmentation and genetic drift can be assumed. This is the first study dealing with three different anthropogenic-induced fragmentation regions. Opposite to predictions, little genetic variation between populations ( $\Phi_{PT}=0.21-0.31$ ) was identified. However, lower values were reported for perennial *Swertia perennis* ( $F_{ST}=0.13$ , Lienert *et al.* 2002) in a setting of one hectare mainland and small isolated remnants (0.5 ha, 1km distance). In *Hypochaeris radicata* populations ( $F_{ST}=0.04$ , Mix *et al.* 2006) sufficient gene flow was detected in a setting of small and large scale fragmentation. Results indicate that fragmentation region (i.e. degree of fragmentation) is of minor influence on genetic variation and eventual inbreeding or genetic drift of populations. A distinct grouping and spatial structure of five, two and six groups has been detected (PcoA, Bayesian analysis) for the fragmentation region Tygerberg, Botteleray and Swartland, respectively. A significant correlation between genetic and geographic distances and isolation by distance effects (Sork *et al.* 1999) were visible for Tygerberg only. Because gene flow was already visible in this region, it should also be present at the less isolated fragments of the Botteleray region. However, due to larger fragment distances and more degraded matrix (i.e. large agricultural areas) that negatively affects movement of pollinators and dispersers, gene flow in Swartland might be reduced.

Habitat fragmentation and subsequent smaller plant populations are susceptible to loss of genetic variation due to genetic drift (Honnay and Jacquemyn 2007) and reduced gene flow (Young *et al.* 1996). This depends on species and site (Jacquemyn *et al.* 2003), as well as on landscape scale (Hutchison and Templeton 1999). If fragmentation effects on population genetic structure are present, they would have been detected (Epperson 1990), especially in annual species with a fragmentation history of over a century. A genetically diverse and regular activated soil seed bank could store much genetic variation (McCue and Holtsford 1998, Muir *et al.* 2004) and buffer against genetic drift (Honnay *et al.* 2008). Although collapse of pollination webs are reported for the region (Pauw 2007), it seems to be likely that pollen and/or seeds are able to bridge *N. barbata* populations, which ensures sufficient gene flow and avoids population differentiation (Slatkin 1985). Seed dispersal is documented in fragmented European grassland species (Honnay *et al.* 2006) where livestock migrated between fragments. The small fruits of *N. barbata* are adapted to gravity-dispersal but could also be dispersed endozoochorous by migrating animals thereby ensuring sufficient gene flow. However, in the case of restricted recent gene flow, observed genetic structure would be an imprint of historic conditions (Templeton 1998). This shows that genetic theory of small populations does not always apply and ecological degradation is more severe for population

persistence than genetic erosion (Kramer *et al.* 2008). Therefore, it is suggested that little impact of fragmentation on genetic variation within and between *N. barbata* populations and regions is currently visible and a panmictic meta-population with random and erratic gene flow and no barrier for pollination and dispersal exists.

## **Conclusion**

*Nemesia barbata* seem to be unaffected by habitat fragmentation effects on population genetics, resulting in rejection of the hypotheses. Substantial genetic variation was found within populations and between populations. No higher genetic variation between Swartland populations was found, a region suffering from most severe fragmentation. This indicates sufficient gene flow in the past and no recent effect of fragmentation. More research is required on genetic variation of the soil seed bank, as well as pollination and dispersal vectors, in order to estimate entire genetic diversity and recent gene flow, respectively. Nevertheless, ongoing habitat transformation will result in population loss and needs to be avoided by the means of habitat protection.

## 8 What determines genetic variation in populations - life history traits or degree of fragmentation?

### Abstract

Land transformation results in fragmented plant populations thereby reducing population sizes and increasing population distances. This habitat fragmentation may be responsible for the decrease of genetic variation in remnant plant populations and higher genetic variation between them. Both, life history traits and degree of fragmentation influence this genetic response; however, studies with both aspects considered are rare. This study tackles this knowledge gap by analyzing renosterveld vegetation of the Cape lowlands in South Africa, a highly fragmented and endangered Mediterranean-type shrubland. Large-scale agricultural land conversion started in the 19<sup>th</sup> century and left only ten percent of renosterveld in small and isolated remnants. Depending on underlying geomorphologic pattern and extent of land conversion, three fragmentation situations with a varying degree of remnant size and isolation are now present in the Cape lowlands: large, medium-distant remnants at the Tygerberg area (hills); semi-large, small-distant remnants at the Botteleray Hills (hill ridge); and small, large-distant remnants at the Swartland (lowland). In each of the regions, six populations of two herbaceous annual Scrophulariaceae, *Hemimeris racemosa* (outcrossing) and *Nemesia barbata* (mixed breeding) were examined. It was argued that fragmentation could have reduced general genetic variation and might be affected by fragmentation degree. Despite the long fragmentation history, AFLP analysis of both species revealed average genetic variation within populations (Nei's gene diversity=0.17, 0.13) and between them ( $\Phi_{ST}$ =0.10, 0.28). Furthermore, low differences of genetic variation between populations (mean  $\Phi_{ST}$ =0.05, 0.26) were detected when three fragmentation regions were analyzed separately. From the data it can be assumed that fragmentation and fragmentation degree do not influence the genetic structure of the species. Furthermore, it seems likely that differences in genetic variation are due to species breeding system rather than fragmentation. Sufficient historical and possibly recent gene flow might mitigate the results of habitat fragmentation.

## Introduction

Anthropogenic induced habitat fragmentation (*sensu* Harrison and Bruna 1999) is coupled with habitat loss (Luijten *et al.* 2000) and smaller population sizes (Oostermeijer *et al.* 1996, Bruna and Kress 2002, Van Rossum *et al.* 2004), thereby increasing isolation of populations (Franklin *et al.* 2002). This will negatively affect biota (Kruess and Tscharntke 1994, Turner 1996, Amler *et al.* 1999) and may result in reduced gene flow (Ouborg *et al.* 2006), inbreeding and drift (Young *et al.* 1996), as well as loss of genetic variation (Fahrig 2001, Honnay *et al.* 2006). Eventually, it can decrease the short- and long-term population viability (Ellstrand and Elam 1993).

Currently, most genetic studies focused on population sizes and compared genetic variation with meta-studies (Hamrick and Godt 1996, Nybom and Bartish 2000). Few have examined whether increasing population distance affects genetic variation between plant population remnants. Both, negative effects (Schmidt and Jensen 2000, Lienert *et al.* 2002) and neutral effects (Foré *et al.* 1992, Young *et al.* 1993) could be observed in this regard. However, such approach lacks calibration of the measured effect. Ideally, fragmentation should be compared to a non-fragmented situation or at least several degrees of fragmentation.

Very few population genetic studies on plant species exist focusing on a multi-fragmentation or multi-species approach (e.g. Foré *et al.* 1992, Young *et al.* 1993, Mix *et al.* 2006) and no study is available dealing with both issues at the same time. This lack was recognised by Ouborg *et al.* (2006) demanding more research on the “rough” edges of population genetics. Specifically, the treatment of population size and isolation as distinct parameters was suggested, as well the incorporation of a multiple species.

Mediterranean-type renosterveld of South Africa is a highly fragmented and endangered shrubland (Rebello *et al.* 2006), offering an opportunity to combine the required multi-fragmentation and multi-species approach. Renosterveld filled once large proportions of the south-western Cape lowlands (Kemper *et al.* 1999), however, mainly agricultural land-transformation destroyed ninety percent of the former extent (von Hase *et al.* 2003a). The dimension of conversion and geomorphologic pattern, such as hills, hill ridge, lowland, resulted in three conspicuously fragmentation regions: large remnants with medium-distant at the Tygerberg area (region A), semi-large remnants with small-distant at the Botteleray Hills (region B), and small remnants with large-distant to neighbour at the Swartland (region C). Plant populations dynamics make it complicated to study plant meta-populations (Ehrlén and Eriksson 2003). This study examined different fragmentation regions to substitute for temporal monitoring of genetic diversity. Such situation is known as space-for-time

substitution (Pickett 1989, Hargrove and Pickering 1992, Travis and Hester 2005, Honnay *et al.* 2009) enabling to compare less fragmented region A and B with the hypothetical result of further habitat loss and isolation in region C. Within this setting, a species-specific detection of strong fragmentation effects on genetic variation of populations and fragmentation regions was hypothesized.

Study species were two annual Scrophulariaceae, namely *Hemimeris racemosa* and *Nemesia barbata*. While *H. racemosa* is outcrossing and found in high abundances (Pauw 2004), *N. barbata* is self-compatible and occurs in low abundances (Datson *et al.* 2006). Genetic variation of a species may be influenced by its rarity. In general, more genetic variation occurs in common species. Although common species are less prone to habitat fragmentation effects (Bijlsma and Loeschcke 2005), it does not seem to have an effect on population differentiation (Hamrick and Godt 1989, Gitzendanner and Soltis 2000, Cole 2003). Many studies deal with population size and genetic variation (Oostermeijer *et al.* 2003) and it seems that the relation is positive and more stronger in self-incompatible than self-compatible plants (Leimu *et al.* 2006). According to Nybom and Bartish (2000), annuals with a mixed breeding system species inherit genetic variation among populations. In general, a positive abundance-occupancy relationship exists, specifically, widespread species tend to be more abundant, whereas more narrow distributed species are less abundant (Gaston *et al.* 2000). Small populations seem to be more prone to genetic drift than larger populations (individuals > 1000) (Montalvo *et al.* 1997). However, opposite patterns are also reported (Mandak *et al.* 2005). Furthermore, genetic variation strongly depends on plant functional traits of the particular species. Higher genetic variability within populations is found in long-lived, woody, outcrossing and late-successional species compared to short-lived, non-woody, self-compatible, and early-successional species, which inherit higher genetic variation between populations (Hamrick and Godt 1996, Nybom and Bartish 2000).

Concerning the general genetic structure, it was aimed to find low genetic variation within and high genetic variation between populations in *H. racemosa*, whereas for *N. barbata* the opposite was suspected. Regarding the impact of fragmentation degree, populations in the three fragmentation regions should inherit gradual genetic variation, depending on fragment size and isolation.

## Material and Methods

### *Species description and sampling procedure*

The study species *Hemimeris racemosa* and *Nemesia barbata* are described in chapter six, page 66 and chapter seven, page 87 respectively. Nevertheless, a brief overview is given in Table 27. Sampling area and procedure was similar as specified in chapter six, page 67 and chapter seven, page 89. It was possible to study genetic variation within and between eighteen populations of *Hemimeris racemosa* and *Nemesia barbata*, as well as between fragmentation regions (Table 28).

**Table 27. Characterization of study species**

Species description follows Goldblatt and Manning (2000). ‡(Olmstead *et al.* 2001). §(Datson *et al.* 2006).

|                     | <i>Hemimeris racemosa</i>                     | <i>Nemesia barbata</i>                                 |
|---------------------|---|--|
| Classification ‡    | Dicotyloid, Scrophulariaceae,<br>Hemimerideae | Dicotyloid, Scrophulariaceae,<br>Hemimerideae          |
| Life form           | Annual  | Annual   |
| Height; leaves      | 40 cm; opposite, ovate and toothed            | 30 cm; opposite, ovate and toothed                     |
| Flower, Flower time | Yellow, double spurred,<br>Jul.-Oct.          | White, blue lower lip, short single spur,<br>Aug.-Oct. |
| Distribution        | Widespread in CFR,<br>high abundances         | Regional in the western CFR,<br>low abundances         |
| Mating system §     | Outcrossing                                   | Mixed breeding system                                  |
| Dispersal mode      | Gravity                                       | Gravity  |

**Table 28. Sampled populations of *Hemimeris racemosa* and *Nemesia barbata*.**

Bold numbers show mean values of each category. † Data following von Hase *et al.* (2003a). \* Data are extracted from aerial photographs.

| Fragmentation region<br>and population | Longitude (E) | Latitude (S) | Fragment size<br>(ha) † | Mean distance to<br>neighboring remnants (km)<br>± S.E |
|--|---------------|--------------|-------------------------|--|
| 1 Tygerberg                            | 18°35'39"     | 33°52'37"    | 595                     | 7.21 ± 1.45  |
| 2 Kanonkop                             | 18°36'16"     | 33°49'35"    | 78                      | 6.31 ± 1.43  |
| 3 Koeberg                              | 18°33'28"     | 33°42'49"    | 141                     | 9.27 ± 1.19  |
| A 4 Porquepine                         | 18°35'15"     | 33°46'10"    | 248                     | 4.38 ± 0.81  |
| 5 Meerendal                            | 18°37'23"     | 33°46'59"    | 298                     | 3.89 ± 0.92  |
| 6 Sondagsfontein                       | 18°39'44"     | 33°45'50"    | 78                      | 6.98 ± 1.18  |
|  |               |              | <b>239.66 ± 79.9</b>    | <b>6.34 ± 0.81</b>                                     |

**Table 28 cont.**

|   |   |               |           |           |                     |                    |
|---|---|---------------|-----------|-----------|---------------------|--------------------|
| B | 1 | Koop PNR      | 18°45'55" | 33°54'14" | 281                 | 1.87 ± 0.46        |
|   | 2 | Zevenwacht    | 18°43'35" | 33°55'16" | 100                 | 3.54 ± 0.91        |
|   | 3 | Mooiplaas     | 18°44'32" | 33°55'29" | 17                  | 2.32 ± 0.70        |
|   | 4 | Wolf bott*    | 18°45'58" | 33°54'53" | 125                 | 1.77 ± 0.59        |
|   | 5 | Wolf top*     | 18°46'15" | 33°55'17" | 125                 | 2.09 ± 0.61        |
|   | 6 | Koop East*    | 18°46'58" | 33°54'04" | 7                   | 4.70 ± 0.78        |
|   |   |               |           |           | <b>94.28 ± 37.3</b> | <b>2.73 ± 0.45</b> |
| C | 1 | Middlepos     | 18°38'37" | 33°40'14" | 4                   | 6.04 ± 1.77        |
|   | 2 | Klipheuwel    | 18°41'23" | 33°41'52" | 52                  | 5.66 ± 1.32        |
|   | 3 | Remshoogte S  | 18°38'55" | 33°38'33" | 20                  | 5.71 ± 1.79        |
|   | 4 | Helderfontein | 18°42'52" | 33°34'03" | 100                 | 11.86 ± 1.11       |
|   | 5 | Remshoogte L* | 18°39'29" | 33°38'51" | 14                  | 5.07 ± 1.74        |
|   | 6 | Klapmuts      | 18°44'45" | 33°44'04" | 34                  | 12.10 ± 1.14       |
|   |   |               |           |           | <b>37.34 ± 14.7</b> | <b>7.74 ± 1.35</b> |

*DNA isolation, AFLP and Statistical analysis*

DNA isolation, AFLP analysis and sequencer procedure are described in detail at chapter six, page 69-72. Selective amplifications were performed with the three primer combinations (Table 29). The statistical analysis of the AFLP data matrix was similar as described in chapter six, page 73-74.

**Table 29. Number of fragments according to primer combination**

| Primer combination | Primer         | <i>Hemimeris racemosa</i> | Loci | <i>Nemesia barbata</i> | Loci |
|--------------------|----------------|---------------------------|------|------------------------|------|
| 1                  | Mse I / Eco RI | +CTC / +AAC               | 85   | +CTA / +ACC            | 76   |
| 2                  | Mse I / Eco RI | +CTC / +AAG               | 91   | +CAC / +AAG            | 63   |
| 3                  | Mse I / Eco RI | +CTG / +ACT               | 96   | +CTC / +ACA            | 67   |
| Total              |                |                           | 272  |                        | 206  |

## Results

### *General genetic structure*

AFLP analyses revealed 272 and 206 fragments for *Hemimeris racemosa* and *Nemesia barbata*, respectively (Table 29). *H. racemosa* and *N. barbata* showed following genetic variation: GD=0.17, 0.13, SI=0.28, 0.20, PL=66 %, 42 %, respectively (Table 30). Lowest genetic variation was found at “*N. barbata* A6”, whereas highest genetic variation was found at “*H. racemosa* A4”. No correlation of fragment size and distance with genetic variation occurred (Table 31). Analyses of molecular variance (Table 32) between populations show low genetic variation in *H. racemosa* (10 %,  $\Phi_{Pt}=0.10$ ) and high genetic variation in *N. barbata* (28 %,  $\Phi_{Pt}=0.28$ ). Bayesian analysis revealed two groups as the most likely number of groupings in *Hemimeris racemosa* and *Nemesia barbata*. In *H. racemosa*, highest  $\Delta K$  was 720.34 with lowest variance of 3.37. In *N. barbata*, highest  $\Delta K$  was 288.09 with lowest variance of Ln P of 2.09 (Table 33). The bar plot shows group allocation for each individual with the highest clustering likelihoods (Fig. 48). *N. barbata* did not show particular grouping, but *H. racemosa* revealed a distinct grouping in fragmentation region B. Mantel test revealed significant isolation by distance for *H. racemosa* (N=292,  $r=0.71$ ,  $p=0.001$ ) but not for *N. barbata* (N=214,  $r=0.15$ ,  $p=0.07$ ).

**Table 30. Genetic variation within populations of *Hemimeris racemosa* and *Nemesia barbata*.** Fragmentation region (Table 28). Sample size (N). Wilcoxon-Test ( $p<0.001$ ) for species comparison of GD ( $Z=3.67$ ), SI ( $Z=3.68$ ) and PL ( $Z=3.72$ ). Significant higher values are depicted by bold numbers.

|                 |        | <i>Hemimeris racemosa</i> |      |      |       | <i>Nemesia barbata</i> |      |       |      |
|-----------------|--------|---------------------------|------|------|-------|------------------------|------|-------|------|
| Fragment region |        | N                         | GD   | SI   | PL    | N                      | GD   | SI    | PL   |
| and population  | number |                           |      |      |       |                        |      |       |      |
| A               | 1      | 14                        | 0.17 | 0.26 | 61.8  | 14                     | 0.13 | 0.209 | 50.5 |
|                 | 2      | 18                        | 0.18 | 0.28 | 68.4  | 7                      | 0.12 | 0.180 | 35.9 |
|                 | 3      | 18                        | 0.18 | 0.29 | 71.3  | 13                     | 0.10 | 0.156 | 35.0 |
|                 | 4      | 19                        | 0.21 | 0.32 | 76.8  | 8                      | 0.11 | 0.167 | 30.1 |
|                 | 5      | 16                        | 0.18 | 0.28 | 73.5  | 12                     | 0.14 | 0.217 | 48.5 |
|                 | 6      | 10                        | 0.15 | 0.24 | 56.3  | 5                      | 0.07 | 0.104 | 20.9 |
| B               | 1      | 20                        | 0.17 | 0.27 | 64.3  | 18                     | 0.13 | 0.194 | 44.2 |
|                 | 2      | 19                        | 0.18 | 0.28 | 72.2  | 20                     | 0.13 | 0.202 | 46.6 |
|                 | 3      | 20                        | 0.16 | 0.25 | 64    | 20                     | 0.14 | 0.217 | 48.5 |
|                 | 4      | 10                        | 0.14 | 0.21 | 47.8  | 5                      | 0.11 | 0.159 | 29.6 |
|                 | 5      | 14                        | 0.16 | 0.25 | 60.3  | 6                      | 0.17 | 0.260 | 49.0 |
|                 | 6      | 13                        | 0.17 | 0.26 | 61.81 | 6                      | 0.11 | 0.169 | 34.5 |

**Table 30 cont.**

|                 |   |    |           |           |          |    |           |           |          |
|-----------------|---|----|-----------|-----------|----------|----|-----------|-----------|----------|
|                 | 1 | 19 | 0.19      | 0.30      | 73.9     | 20 | 0.15      | 0.24      | 53.40    |
|                 | 2 | 20 | 0.19      | 0.30      | 79.8     | 20 | 0.17      | 0.26      | 59.71    |
|                 | 3 | 18 | 0.18      | 0.28      | 70.2     | 20 | 0.16      | 0.24      | 51.46    |
| C               | 4 | 8  | 0.16      | 0.25      | 53       | 10 | 0.13      | 0.21      | 45.15    |
|                 | 5 | 18 | 0.19      | 0.29      | 71.3     | 7  | 0.16      | 0.24      | 44.66    |
|                 | 6 | 18 | 0.18      | 0.28      | 69.9     | 3  | 0.10      | 0.15      | 27.18    |
| All populations |   |    | 0.17±0.01 | 0.27±0.01 | 66.5±2.0 |    | 0.13±0.01 | 0.20±0.01 | 41.9±2.5 |

**Table 31. Pearson correlation coefficient of fragment size and distance with genetic variation.**  
Significant correlations did not occur ( $p < 0.05$ ).

|                    | Correlation of fragment size with |        |        | Correlation of mean distance to neighboring remnants with |        |        |
|--------------------|-----------------------------------|--------|--------|---|--------|--------|
|                    | GD                                | SI     | PL     | GD  | SI     | PL     |
| <i>H. racemosa</i> | 0.000                             | -0.032 | -0.079 | 0.133   | 0.180  | 0.033  |
| <i>N. barbata</i>  | -0.084                            | -0.045 | 0.096  | -0.332  | -0.272 | -0.222 |

**Table 32. Analysis of molecular variance of *Hemimeris racemosa* and *Nemesia barbata*.**

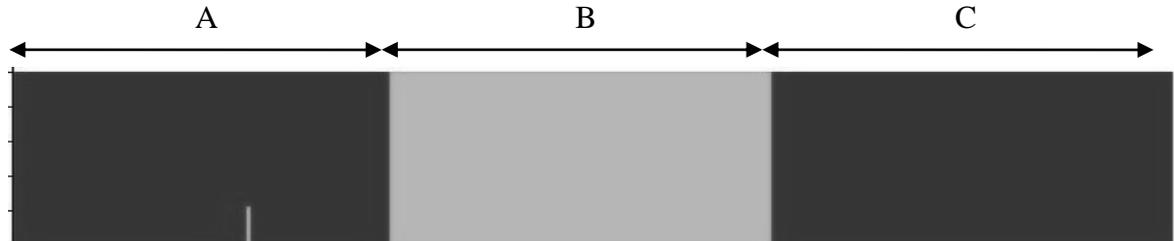
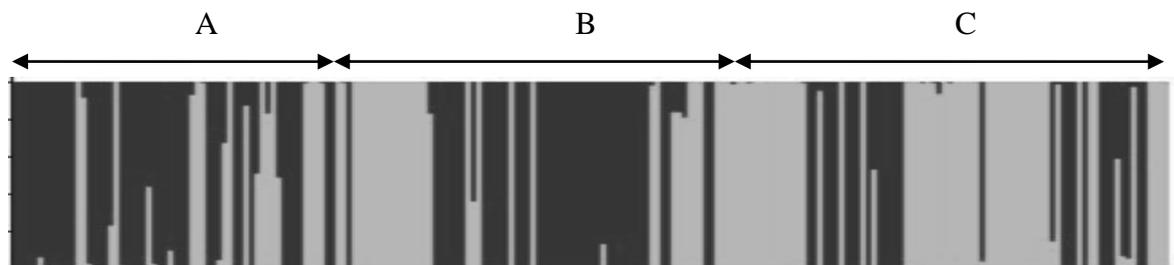
Individuals (I), Populations (N). Based on AFLP fragments. Degrees of freedom (df). Proportion of genetic variation (%).  $F_{St} = \Phi_{Pt}$ . Significance level ( $p > 0.001$ ) is based on 999 permutations.

| Species            | I   | N  | Loci | Genetic variation   | df  | Sums of squares | Means squares | %  | $\Phi_{Pt}$ |
|--------------------|-----|----|------|---------------------|-----|-----------------|---------------|----|-------------|
| <i>H. racemosa</i> | 292 | 18 | 272  | Between populations | 17  | 1453.73         | 85.51         | 10 | 0.10        |
|                    |     |    |      | Within populations  | 274 | 8127.01         | 29.66         | 90 |             |
| <i>N. barbata</i>  | 214 | 18 | 206  | Between populations | 17  | 1549.86         | 91.17         | 28 | 0.28        |
|                    |     |    |      | Within populations  | 196 | 3208.81         | 16.37         | 72 |             |

**Table 33. Bayesian model-based clustering likelihoods and model selection.**

True number of groups ( $k$ ) and log probability of data  $\ln P(D)$ . Ad hoc quantity based on rate of change of likelihood function with respect to  $K$  calculation ( $\Delta K$ ). 10 replicate runs. Values of  $K > 10$  are not reported due to low likelihood. Model selection for number of groups ( $k$ ) present in the dataset. Calculation based on 272 AFLP fragments for 292 *Hemimeris racemosa* and 206 AFLP fragments for 214 *Nemesia barbata* individuals from 18 populations. ‡Model selection method (Evanno *et al.* 2005).

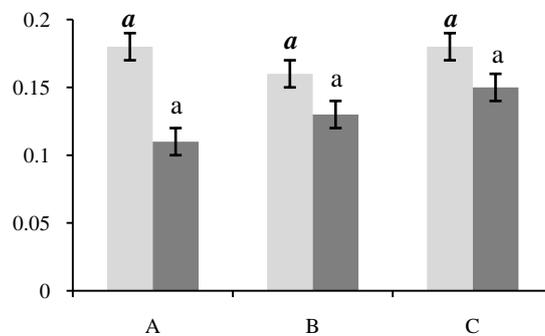
| k  | <i>Hemimeris racemosa</i> |        |           |               | <i>Nemesia barbata</i> |         |             |               |
|----|---------------------------|--------|-----------|---------------|------------------------|---------|-------------|---------------|
|    | Mean $\ln P(D)$           | S.E.   | Variance  | $\Delta k$ ‡  | Mean $\ln P(D)$        | S.E.    | Variance    | $\Delta k$ ‡  |
| 1  | -30574.59                 | 0.47   | 2.17      |               | -15131.41              | 0.15    | 0.23        |               |
| 2  | -28885.71                 | 0.58   | 3.37      | <b>720.34</b> | -14201.99              | 0.46    | 2.09        | <b>288.09</b> |
| 3  | -28519.62                 | 1.21   | 14.73     | 76.49         | -13689.49              | 5.88    | 345.36      | 5.12          |
| 4  | -28447.13                 | 11.76  | 1383.26   | 1.96          | -13272.22              | 23.17   | 5367.74     | 1.98          |
| 5  | -28353.34                 | 21.58  | 4657.61   | 2.38          | -12918.18              | 23.37   | 5460.02     | 22.78         |
| 6  | -28400.85                 | 73.58  | 54140.80  | 2.01          | -14197.24              | 1518.92 | 23071213.48 | 0.65          |
| 7  | -28524.07                 | 137.50 | 189053.04 | 1.13          | -12461.66              | 14.98   | 2244.68     | 45.24         |
| 8  | -28724.97                 | 263.60 | 694861.93 | 1.27          | -12841.14              | 351.65  | 1236582.51  | 1.59          |
| 9  | -28974.17                 | 292.15 | 853517.02 | 1.72          | -12671.41              | 506.51  | 2565558.14  | 0.98          |
| 10 | -28900.33                 | 166.93 | 278654.40 | 2.83          | -12910.88              | 456.85  | 2087137.34  | 1.06          |

*Hemimeris racemosa**Nemesia barbata***Fig. 48. Individual assignments to  $K=2$  demes.**

Letters indicate fragmentation region A, B and C according to Table 28.

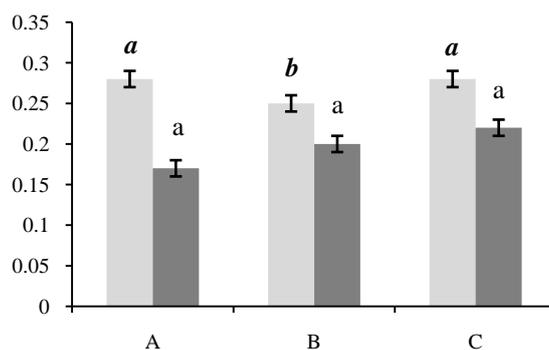
*Impact of fragmentation degree on genetic structure*

*Hemimeris racemosa* revealed higher genetic variation within populations in all fragmentation regions compared to *Nemesia barbata* (Fig. 49, Fig. 50, Fig. 51). Within each species, no differences of genetic variation within populations occurred between fragmentation regions, except for significantly lower Shannon's Information Index in region B for *Hemimeris racemosa*. A comparison of fragmentation regions (Table 34) in *H. racemosa* showed less genetic variation between populations ( $\Phi_{iPt} = \sim 0.05$ ) compared to *N. barbata* ( $\Phi_{iPt} = 0.21-0.36$ ). Bayesian analysis of single fragmentation regions revealed several groups as the most likely number of groupings in *Hemimeris racemosa* and *Nemesia barbata* (Table 35). In *H. racemosa*, highest  $\Delta K$  and most likely clustering was two groups in fragmentation region A ( $k=20.67$ ) and B ( $k=11.34$ ), and five groups in region C ( $k=4.09$ ). In *N. barbata*, highest  $\Delta K$  and most likely clustering was five groups in fragmentation region A ( $k=10.75$ ), two groups in region B ( $k=144.07$ ), and four groups in region C ( $k=6.74$ ). Whereas Mantel test was not significant for *N. barbata*, it revealed significant isolation by distance for *H. racemosa* and within fragmentation region B and C (Table 36).

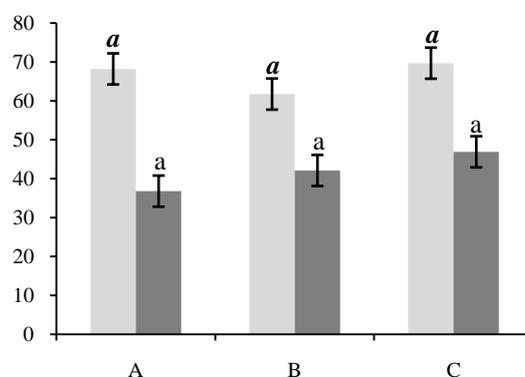


**Fig. 49. Nei's Gene diversity of *Nemesia barbata* and *Hemimeris racemosa*.**

Nei's gene diversity  $\pm$  S.E. Fragmentation region according to Table 28. ANOVA ( $p < 0.05$ ) for group comparison in *Hemimeris racemosa* (light grey,  $F=2.45$ ) and in *Nemesia barbata* (dark grey,  $F=2.84$ ). Significant region comparison is depicted by different letters. Wilcoxon-Test ( $p < 0.05$ ) for species comparison at fragmentation region level  $Z=2.20$ . Significant higher values are depicted by bold and italic letters.



**Fig. 50. Shannon's Information Index of *Nemesia barbata* and *Hemimeris racemosa*.** Shannon's Information index  $\pm$  S.E. Fragmentation region according to Table 28. ANOVA ( $p < 0.05$ ) for group comparison in *Hemimeris racemosa* (light grey,  $F=2.78$ ) and in *Nemesia barbata* (dark grey,  $F=2.61$ ). Significant region comparison is depicted by different letters. Wilcoxon-Test ( $p < 0.05$ ) for species comparison at fragmentation region level  $Z=1.99$ . Significant higher values are depicted by bold and italic letters.



**Fig. 51. Percentage of polymorphic loci of *Nemesia barbata* and *Hemimeris racemosa*.** Percentage of polymorphic loci  $\pm$  S.E. Fragmentation region according to Table 28. ANOVA ( $p < 0.05$ ) for group comparison in *Hemimeris racemosa* (light grey,  $F=1.56$ ) and in *Nemesia barbata* (dark grey,  $F=1.46$ ). Significant region comparison is depicted by different letters. Wilcoxon-Test ( $p < 0.05$ ) for species comparison at fragmentation region level  $Z=2.20$ . Significant higher values are depicted by bold and italic letters.

**Table 34. Analysis of molecular variance of *Hemimeris racemosa* and *Nemesia barbata*.**

Based on AFLP fragments (loci). Degrees of freedom (df). Proportion of genetic variation (%). Significance level ( $p > 0.001$ ) is based on 999 permutations. *Hemimeris racemosa* (*Hr*). *Nemesia barbata* (*Nb*).

| Species   | Region | Individuals | Populations | Loci | Genetic variation   | df | Sums of squares | Means squares | %  | Phi <sub>Pt</sub> |
|-----------|--------|-------------|-------------|------|---------------------|----|-----------------|---------------|----|-------------------|
| <i>Hr</i> | A      | 95          |             |      | Between populations | 5  | 267.86          | 53.57         | 5  | 0.05              |
|           |        |             |             |      | Within populations  | 89 | 2687.25         | 30.19         | 95 |                   |
|           | B      | 95          | 6           | 272  | Between populations | 5  | 245.30          | 49.06         | 5  | 0.05              |
|           |        |             |             |      | Within populations  | 90 | 2447.38         | 27.19         | 95 |                   |
|           | C      | 101         |             |      | Between populations | 5  | 251.39          | 50.28         | 3  | 0.04              |
|           |        |             |             |      | Within populations  | 95 | 2992.37         | 31.50         | 97 |                   |
| <i>Nb</i> | A      | 59          |             |      | Between populations | 5  | 315.67          | 63.13         | 26 | 0.26              |
|           |        |             |             |      | Within populations  | 53 | 772.91          | 14.58         | 74 |                   |
|           | B      | 75          | 6           | 206  | Between populations | 5  | 466.73          | 93.35         | 31 | 0.31              |
|           |        |             |             |      | Within populations  | 69 | 1043.59         | 15.12         | 69 |                   |
|           | C      | 80          |             |      | Between populations | 5  | 410.16          | 82.03         | 21 | 0.21              |
|           |        |             |             |      | Within populations  | 74 | 1392.31         | 18.82         | 79 |                   |

**Table 35. Bayesian model-based clustering likelihoods and model selection.**

$\Delta k$  with model selection method (Evanno *et al.* 2005). 10 replicate runs. Model selection for number of groups ( $k+2$ ) present in the dataset. *Hemimeris racemosa* (*Hr*). *Nemesia barbata* (*Nb*).

| Runs (k) | Fragmentation region and species |              |              |               |             |             |
|----------|----------------------------------|--------------|--------------|---------------|-------------|-------------|
|          | A                                |              | B            |               | C           |             |
|          | <i>Hr</i>                        | <i>Nb</i>    | <i>Hr</i>    | <i>Nb</i>     | <i>Hr</i>   | <i>Nb</i>   |
| 2        | <b>20.67</b>                     | 3.13         | <b>11.34</b> | <b>144.07</b> | 3.49        | 2.71        |
| 3        | 1.04                             | 3.02         | 5.83         | 4.78          | 2.09        | 2.91        |
| 4        | 3.19                             | 1.74         | 1.25         | 2.76          | 1.21        | <b>6.74</b> |
| 5        | 1.22                             | <b>10.75</b> | 1.73         | 1.27          | <b>4.09</b> | 1.34        |
| 6        | 2.16                             | 1.67         | 1.66         | 6.64          | 0.89        | 1.11        |
| 7        | 2.69                             | 2.78         | 1.84         | 4.26          | 2.06        | 4.27        |
| 8        | 1.08                             | 0.87         | 0.68         | 0.51          | 1.04        | 2.09        |

**Table 36. Mantel test with correlation coefficient of genetic and geographical distances.**

Bold numbers show significant correlations between genetic distances ( $\Phi_{iTP}$ ) and Geographical distances (km).

| Fragmentation region | <i>Hemimeris racemosa</i> |       |              | <i>Nemesia barbata</i> |      |      |
|----------------------|---------------------------|-------|--------------|------------------------|------|------|
|                      | N                         | r     | p            | N                      | r    | p    |
| A                    | 95                        | -0.36 | 0.15         | 59                     | 0.54 | 0.07 |
| B                    | 96                        | 0.77  | <b>0.002</b> | 75                     | 0.21 | 0.23 |
| C                    | 101                       | 0.86  | <b>0.004</b> | 80                     | 0.25 | 0.25 |

## Discussion

Currently, multi-species approaches within a fragmentation context are only known from zoological studies (e.g. Bates 2002). In order to characterize possible fragmentation effects on two plant species, 292 *Hemimeris racemosa* and 214 *Nemesia barbata* individuals were collected from 18 renosterveld remnants. In the analysis of genetic variation a large number of polymorphic DNA fragments have been effectively detected for both species, 272 and 206 loci, respectively.

Specifically, it was asked if low genetic variation within populations and high genetic variation between them would be visible for outcrossing *H. racemosa*. The opposite was suspected for mixed breeding *N. barbata*. Furthermore, gradual genetic variation between regions was conjectured, depending on fragment size and isolation. Contrary to predictions, results indicated average genetic variation within and between populations and fragmentation regions for a both species. No significant correlation between genetic variation and fragment size nor distance to neighbour fragment was visible.

*General genetic structure*

In *H. racemosa* significant higher genetic variation within populations was observed (GD=0.17, SI=0.27, PL=66 %), compared to *N. barbata* (GD=0.13, SI=0.20, and PL=42 %). However, genetic variation within populations was similar to previous studies on outcrossing, gravity-dispersed and early succession species compared to mixed breeding species (Hamrick and Godt 1996, Nybom and Bartish 2000). Genetic variation within populations is of considerable and average magnitude; hence, habitat fragmentation seems not to have caused genetic drift and inbreeding in the generation cycles during fragmentation. In *H. racemosa* lower genetic variation between populations ( $\Phi_{PT}=0.10$ ) was observed, than in *N. barbata* ( $\Phi_{PT}=0.28$ ). Regarding *H. racemosa*, similar values were found in studies that focussed on population distances smaller than 20 km (Hooftman *et al.* 2004, Van Rossum *et al.* 2004, Leimu and Mutikainen 2005, Honnay *et al.* 2006). The values in these studies were much smaller than results observed in *N. barbata*, but consistent with data for gravity-dispersed species with mixed breeding system (Hamrick and Godt 1996, Nybom and Bartish 2000).

Outcrossing species, such as *H. racemosa*, retain high genetic variation within populations, whereas mixed breeding species (e.g. *N. barbata*), show higher genetic variation between populations (Hamrick and Godt 1996, Nybom and Bartish 2000). Therefore, the different genetic variation between populations of both species might be entirely due to the breeding system, rather than possible fragmentation effects and subsequent genetic drift and inbreeding. This is supported by a meta-study of Cole (2003) where no effect occurred between rare and common plant populations because “similarity of breeding system in congeneric species”. Hence, similarity or dissimilarity seems to be an overriding factor in distribution of genetic variation.

In the same meta-study it was found that rare plants had lower levels of genetic variation within populations than common plants. This is in line with a comparative study in fynbos vegetation, that has found higher genetic variation within populations and lower genetic differentiation in the common *Mimetes fimbriifolius* than the more rare *M. hirtus* (Reisch *et al.* 2010). Nevertheless, negative effects of habitat fragmentation can be more pronounced for formerly common and recently declining species and populations, than for naturally rare species and populations (Huenneke 1991). This is also stated for rare renosterveld plants species that could be unaffected by inbreeding depression (Rebelo 1992a) and extinction processes (Kemper *et al.* 1999).

The effect of population size on genetic variation is well studied and most agree that small populations are more prone to genetic drift than larger populations were genetic drift becomes

a minor factor (Montalvo *et al.* 1997). However, no such relation was found in *H. racemosa* and *N. barbata*. Bayesian analysis revealed two groups as the most likely number of groupings in both species. As shown this is due to the separating Botteleray region (*H. racemosa*, chapter six) and one outlier group (*N. barbata*, chapter seven), which is contrary to the predictions of a distinct grouping of the Swartland region. A significant correlation between genetic and geographic distances occurs for *H. racemosa*, but not for *N. barbata*. In the case of *H. racemosa*, this can be due to isolation by distance effects (Sork *et al.* 1999) and indicates a sufficient gene flow.

#### *Impact of fragmentation degree on genetic structure*

Population genetics studies are often of restricted explanatory power because of the challenge to compare recent fragmentation with the previous situation. The disadvantage could be avoided because it was possible to calibrate the fragmentation effect using space-for-time substitution (*sensu* Pickett 1989). Very few studies with similar complex fragmentation setting exist so far. Previous studies have mainly examined population size assuming similar degree of isolation for all populations. This is the first attempt to study three different fragmentation settings induced by anthropogenic habitat transformation. Despite the advantageous setting, no gradual effect of fragmentation degree on genetic variation was found.

Within each species, no significant differences of genetic variation within populations occurred between fragmentation regions, except for significant lower Shannon's Information Index in region B for *Hemimeris racemosa*. Hence, no reduction in genetic variation within populations occurred in the highly fragmented Swartland region. Therefore, minor impact of fragmentation on genetic variation and little genetic drift can be assumed. *H. racemosa* has always shown significant higher genetic variation than *N. barbata*. This situation was already interpreted as a result of their different breeding system (above).

Minor differences in genetic variation between populations of *H. racemosa* ( $\Phi_{PT}=0.05$ , 0.05, 0.04) were identified between fragmentation regions. Similar values and sufficient gene flow were reported in a setting of small- and large-scale fragmentation (Mix *et al.* 2006), as well as for isolated local populations where higher wind impact enhanced dispersal rates and reduced genetic differentiation (Foré *et al.* 1992, Young *et al.* 1993). In contrast, population differentiation was visible in perennial *Swertia perennis* ( $F_{ST}=0.13$ , Lienert *et al.* 2002), occurring at one hectare mainland and small isolated remnants (0.5 ha, 1km distance).

At regional scale, minor differences in genetic variation between populations were identified for *N. barbata* ( $\Phi_{PT}=0.26, 0.31, 0.21$ ). In contrast to the predictions, lowest differentiation occurred in the smallest and most isolated populations of the Swartland and is possibly due to gene flow via livestock or game. The high differentiation at Botteleray ( $\Phi_{PT}=0.31$ ) could be explained with the high proportion of pine plantation between fragments that could act as an effective gene flow barrier for pollinators and dispersal vectors.

The genetic constitution of *H. racemosa* and *N. barbata* indicates that fragmentation region (i.e. fragmentation degree) is of minor influence regarding genetic variation between populations, and possible inbreeding or genetic drift. Bayesian analysis of single fragmentation regions revealed several numbers as the most likely groupings in both species. In *H. racemosa*, two groups in fragmentation region A and B, and five groups in region C were identified. In *N. barbata*, five groups in fragmentation region A, two groups in region B and four groups in region C were identified.

A global Mantel test was not significant for any region of *N. barbata* and gene flow may therefore be reduced. Significant isolation by distance (Sork *et al.* 1999) was revealed for *H. racemosa* within fragmentation region B and C. For *H. racemosa* at Tygerberg region, the idea of non-existing gene flow is rejected because gene flow was already visible in small and isolated Swartland fragments giving no reason why gene flow processes should not be present in larger and less isolated fragments of the Tygerberg region.

Habitat fragmentation can negatively influence populations and gene flow (Young *et al.* 1996). Subsequent smaller plant populations are susceptible to extinction due to loss of genetic variation via genetic drift and mating among related individuals (Honnay and Jacquemyn 2007). These alterations may depend on landscape scale (Hutchison and Templeton 1999) and can be species- and site-specific (Jacquemyn *et al.* 2003). Genetic theory of small populations does not always apply and ecological degradation is more important than genetic degradation at least for well dispersed species (Kramer *et al.* 2008). A lack of consistency in effects of habitat fragmentation is also reported by Debinski and Holt (2000). Study results support the latter ideas that species are more threatened by habitat loss than by genetic erosion.

Results show little effects of fragmentation degree on genetic variation, which are promising news for both species. It seems to be likely that one pollen or seed per generation is able to bridge populations, which is necessary to ensure sufficient gene flow and avoiding population differentiation (Slatkin 1985). Although collapse of pollination webs are reported for the urban areas (Pauw 2007), it seems that pollinators are still able to move between non-urban

populations. The further vector for gene flow is a diaspore (Honnay *et al.* 2006, Mix *et al.* 2006). *H. racemosa* and *N. barbata* have small fruits without attachments for anemochorous or exozoochorous dispersal, but endozoochours dispersal by migrating animals could ensure sufficient gene flow.

Time span could have been too short to measure fragmentation effects on population genetic structure. In this case the observed pattern would be an imprint of historic conditions with high gene flow (Templeton 1998). For example, a steadily activated and genetically diverse seed bank could store much genetic variation (McCue and Holtsford 1998, Muir *et al.* 2004) and might buffer against genetic drift and differentiation (Honnay *et al.* 2008). However, this can be excluded for both annuals because fragmentation history dates back over a century and differentiation is already detectable after few generations (Epperson 1990). Therefore, it is suggested that little impact of fragmentation on genetic variation within and between *H. racemosa* and *N. barbata* populations and regions is currently visible and a panmictic meta-population with random and erratic gene flow and no barrier for pollination and dispersal exists.

## 9 Summary

The main aim of the thesis was to reveal consequences of habitat degradation and fragmentation in renosterveld vegetation. The first part of the thesis focused on renosterveld degradation. In order to estimate the ecosystem health and restoration potential, pristine and degraded renosterveld habitats were compared using vegetation, soil and soil seed bank (chapter 2). Additionally, the influence of smoke on germination of soil seed bank was tested (chapter 3). Invasive plant species are part of the degradation problem and therefore germination behavior of the most problematic alien species in renosterveld - *Echium plantagineum* - was examined (chapter 4). Furthermore, restoration experiments were carried out to show restoration possibilities and priorities (chapter 5). The second part of the thesis concentrated on the consequences of habitat fragmentation on genetic variation of plant populations. The genetic structure of two Scrophulariaceae was analyzed (chapter 6-7).

More specifically, in chapter one, it was evident that renosterveld research is of relatively recent but intense nature compared to fynbos vegetation that has received much more attention. The reason is the suitability of fertile renosterveld soils for agriculture, leaving the vegetation highly transformed and fragmented since the beginning of European settlement. Much effort is now pointed to restoring this species-rich vegetation type. Unfortunately, restoration attempts of abandoned fields until now lacked success and new approaches are needed to manage those sites. Furthermore, pines stands expanding rapidly in the region, but have been overlooked in scientifically monitored restorations schemes. In chapter two, the extent of degradation on vegetation, soil and soil seed bank was evaluated in pristine and degraded renosterveld. A minor restoration potential of abandoned fields was detected, due to depletion of indigenous soil seed bank, nutrient enrichment, and high cover and competition of alien grasses, especially *Avena barbata*, *Bromus pectinatus* and *Lolium multiflorum*. In contrast, the restoration potential appears to be high with pine plantation due to its viable seed bank, lower soil alteration and less alien species. In fynbos vegetation it was found that smoke (i.e. fire surrogate) was the main trigger for the germination of most species. In chapter three, the effect of smoke primer on soil seed banks in renosterveld was evaluated. Findings suggest that smoke-primer has a lower effect on seeds of renosterveld species compared to fynbos species. This could be a new argument in the question of renosterveld origin. Maybe renosterveld was indeed more a herbivore-prone grassland than a fire-prone shrubland. Overall fire should play a role in renosterveld management to ensure the germination and establishment of rare species. However, caution is needed because smoke-primer can favour invasive alien species, such as *Echium plantagineum*, that are stored in the soil seed bank. In

chapter four, adaptations of the germination response of problematic alien *Echium plantagineum* in regard to smoke-primer was tested. It was observed that germination plasticity to smoke-primer treatment exist, which is depending on seed origin and growth habitat. Higher germination response was visible in South African roadside populations that are subjected to regular human-caused fires, compared to populations from natural habitats, as well as from French and Australian populations. In chapter five, restoration experiments were carried out to assess the possibilities of re-establishment of indigenous shrub matrix into degraded renosterveld vegetation. A pine clearing initiated by the local nature conservation authorities resulted in great recovery of indigenous plant species. Hence pine clearings seem to be a promising strategy to re-activate indigenous plant species from the soil seed bank. Experiments with *Otholobium hirtum* (pioneer shrub species, sowing approach) and bird-dispersed plants (termitaria species, perch approach) in abandoned fields showed minor establishment success, despite successful pre-testing. Before launching other large-scale restoration programs, pre-testing is strongly recommended without a guarantee for success.

In chapter six, the influence of habitat fragmentation on genetic variation of *Hemimeris racemosa* population was studied. Results revealed high genetic variation within and a low genetic variation between populations and fragmentation regions. These pattern and values of genetic variation are typical for outbreeding plants. Therefore, it can be assumed that a minor effect of fragmentation is present yet, with sufficiently large populations to conserve all genetic variability and a reasonable gene flow. In chapter seven, the impact of fragmentation on genetic variation of *Nemesia barbata* populations was evaluated. Low genetic variation within and high genetic variation between populations were revealed, and in line with meta-data from mixed-breeding plants. In this species only a minor effect of fragmentation is present yet. In chapter eight, genetic variation of *Hemimeris racemosa* and *Nemesia barbata* populations from exactly the same fragments were compared. Again, it was observed that genetic variation of fragmented populations depend more on breeding system and not on fragmentation degree as suspected.

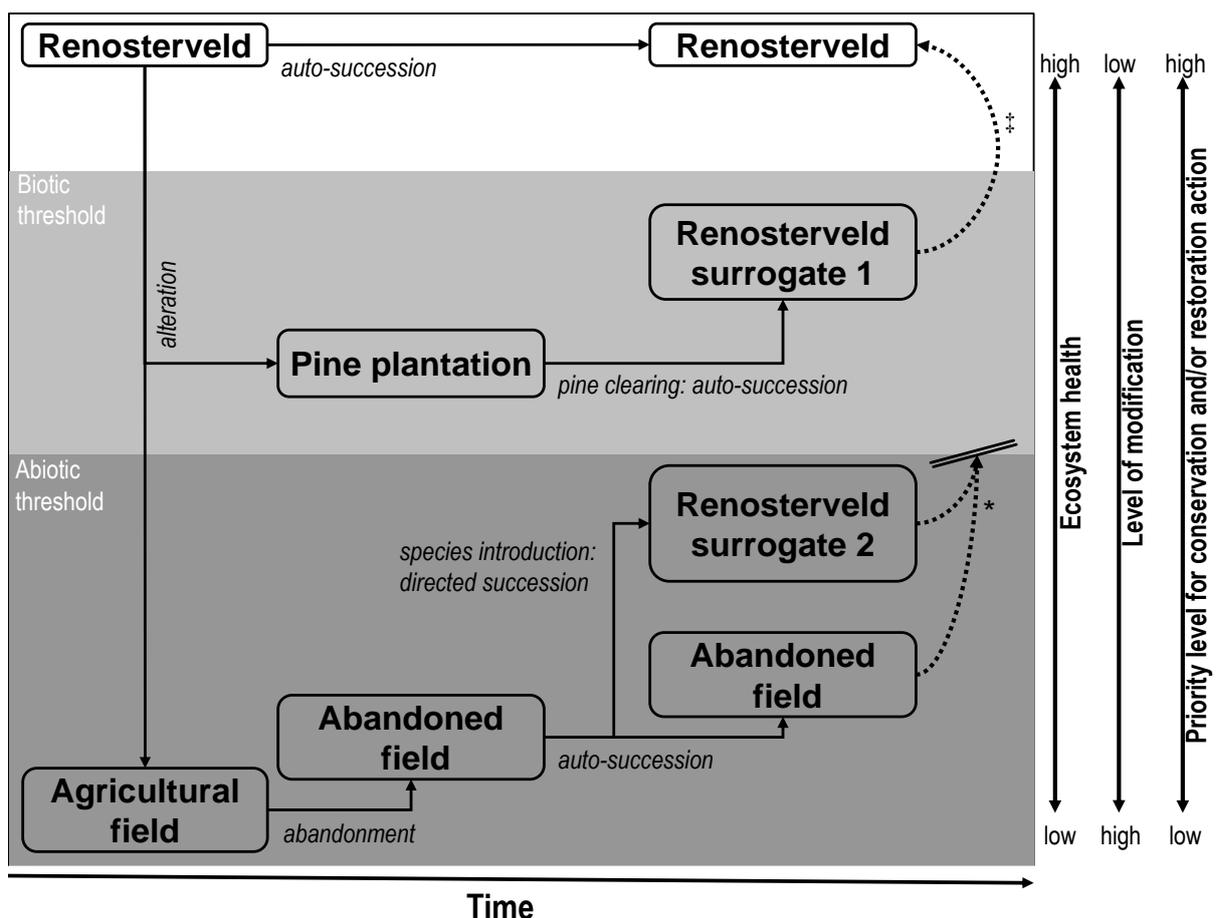
In summary, conclusions from thesis findings are that recent conservation and restoration attempts in renosterveld need to be adjusted towards allocation of efforts and resources to existing remnants with focus on habitat protection and easy-to-restore pine plantations. This also makes sense because genetic variation seems not to be impacted yet by habitat fragmentation. Degraded habitats with dominant alien grass, such as abandoned fields, should be of the least concern with auto-succession being recommended. Despite its low ecosystem health, degraded sites provide ecosystem services, such as erosion protection and feeding

ground for antelopes, and are termed novel ecosystems (Lugo 1992, Hobbs et al. 2006, Marris 2009). If any restoration attempts are being made here, they should work towards a shrub state or focus on competitive geophytes in order to suppress alien grasses. Alternatively, alien grass areas could serve as sites 'to reach a specific objective' (Boucher 1995), such as nurseries of threatened, overused, medicinal or horticultural plant species. The degree of genetic variation of *Hemimeris racemosa* and *Nemesia barbata* populations do not seem to depend on fragmentation or fragmentation degree as suspected. This is a promising result for the persistence of the studied species and a strong argument to safe pristine renosterveld sites. Presented recommendations are best expressed with:

- *Safe pristine renosterveld*
- *Clear pine stands*
- *Eventually benefit from abandoned fields*

## 10 Perspectives

The global change of land use, climate, nitrogen deposition and carbon dioxide levels will alter ecosystems and biodiversity in the near future (Sala et al. 2000). These impacts and their uncertain interactions will also affect Mediterranean-type ecosystems and therefore renosterveld management. Degraded habitats and novel ecosystem show difficulties to reach historical conditions (Lugo 1992, Hobbs et al. 2006, Cramer et al. 2008, Marris 2009). Therefore, future restoration goals should balance restoration options and costs, focusing on the characteristics of future ecosystems and avoiding to preserve a historical status (Hobbs and Harris 2001). Based on the study results, a model for renosterveld succession and management is proposed (Fig. 52) and explained below. It could frame future attempts in renosterveld research.



**Fig. 52. Renosterveld succession and management model.**

Partly adapted from Whisenant (1999), Hobbs and Harris (2001), Suding, LeJeune *et al.* (2004).

Every piece of pristine renosterveld counts and existing efforts should be guided into prevention of further loss of habitat and natural capital (Aronson *et al.* 2006a, Aronson *et al.* 2006b). For example, renosterveld provides important hydrological ecosystem services (O'Farrell and Collard 2003, O'Farrell *et al.* 2009) and may improve livestock health (Kemper *et al.* 1999). On-farm conservation (Kemper *et al.* 1999) and promotion of those services (O'Farrell *et al.* 2009) are of high priority. In general, renosterveld conservation is at relatively low cost compared to its regional and global importance (Frazee *et al.* 2003). Future restoration and conservation investments should be guided into immediate action (Cowling *et al.* 2010).

Alteration of pristine renosterveld is due to conversion into pine plantations and agricultural land, thereby passing biotic and abiotic thresholds, respectively. The regenerative potential of a pine plantation site was demonstrated in this study (chapter two, three and five, this thesis). The intact renosterveld soil seed bank was still viable under pines and indigenous species recovered quickly after pine clearing. This restoration potential was also acknowledged by C. Boucher (unpubl. data) and De Villiers *et al.* (2005). Because of increasing habitat deterioration it is crucial to highlight the benefits of such restoration (Aronson *et al.* 2010). For example, pine clearing enhances ecosystem services, such as higher water flow and lower fire hazard (Le Maitre *et al.* 2002). In general, the post-clearing site will follow succession by activating the viable seed bank, as well as allowing for more recruitment of dispersed diaspores from adjacent pristine renosterveld due to the reduced pine cover and litter. Finally, the pine-cleared site will develop into a renosterveld surrogate 1, which has the potential to reach a true renosterveld status (see ‡, Fig. 52), if further succession and a fire/herbivore disturbance regime is taking place.

Renosterveld conversion by means of agriculture passes biotic *and* abiotic thresholds, leaving very little chance of succession into a renosterveld surrogate after abandonment. Abandoned agricultural fields are a common feature of the Cape Lowlands and initial restoration attempts have been made. However, studies show that restoration potential is low because of heavily degraded abiotic conditions, limited seed influx and alien grass competition (chapter two, this thesis; Krug and Krug 2007). Invasibility into dominant vegetation is depending on species (Emery and Gross 2006) and seem to be poor for abandoned fields (Krug and Krug 2007). Several restoration studies have reported similar difficulties in re-establishing indigenous species into abandoned fields in the Mediterranean Basin (Römermann *et al.* 2005, Buisson *et al.* 2006, Pueyo and Alados 2007), Californian chaparral (Cione *et al.* 2002, Mau-Crimmins 2007) and Australian wheat belt (Standish *et al.* 2007). Such abandoned fields are beyond

biotic and abiotic thresholds and neither improved management nor vegetation manipulation can help to change succession direction (Stylinski and Allen 1999, Hobbs and Cramer 2007b). Abandoned fields of renosterveld in the western Cape Lowlands of South Africa, and the studied sites at Tygerberg Nature Reserve in particular, seem to have suffered similar problems and show little recovery towards a historic vegetation state because the mentioned thresholds have been passed (Krug and Krug 2007). It is of doubt that changes of these thresholds would be successful because immense restoration management and efforts would be required (*sensu* Hobbs and Harris 2001) and currently no promising large-scale restoration method is available (Krug and Krug 2007). This situation leaves manual species introduction (directed succession) as the most appropriate, but very expensive method. It is questionable if those sites (abandoned fields, renosterveld surrogate 1) will ever reach renosterveld-like state (see \*, Fig. 52).

How to deal with abandoned fields in the long-term? Certainly, novel restoration and management approaches are needed to improve ecosystem health of abandoned fields. However, such attempts are difficult and can easily fail (e.g. chapter five, this thesis). Even promising tools like fire surrogates have not proved successful and are difficult concerning favouring alien species (e.g. chapter three and four, this thesis). Abandoned fields should either be left in their quasi-steady-state or made available for novel plant communities to achieve specific objectives (Boucher 1995). Alternative uses are manifold, such as the horticultural and medicinal sector offering a sustainable use of target species (Geldenhuis 2002) and various income-generating products (Kruger 1982). Establishment of nursery habitats for endangered and medicinal plant species could provide valuable and much needed socio-economic benefits. Indeed, the Cape region shows an increasing demand of medicinal plants products (Loundou 2008, Lourens *et al.* 2008), which means renosterveld plant populations have been severely over-utilized in the past (Naidoo 1994, McKenzie *et al.* 1995, Moerat 1995). In order to avoid population extinction and to enhance restoration efforts of abandoned fields, the sites could function as nursery locations of overused medicinal, edible, horticultural (Powrie 1995) and highly threatened renosterveld plant species. Such attempt could support capacity building and provide sustainable income of under-privileged community members. Cultivation of such plants could also potentially support pollinators, thus maintaining a pollination network that offers another ecosystem service in the landscape. Although they do not fit biodiversity strategies, such attempts might be an useful approach for private sector (P. Holmes, pers. comm.).

With this in mind, the renosterveld model can therefore be summarized:

*Safe pristine renosterveld, Clear pine stands, and Eventually benefit from abandoned fields.*

This scheme and the different levels of ecosystem health provide a point of orientation for conservation priority and restoration potential (Table 37). Existing efforts and resources should hence focus on prevention of further renosterveld habitat loss, which again are the most urgent and main conservation priority. Pine plantations show a medium ecosystem health and therefore high restoration potential and priority. Abandoned fields are of less concern and have low ecosystem health. They have a low restoration potential and priority.

**Table 37. Ecosystem health, conservation and restoration of different renosterveld habitats.**

<sup>#</sup> derived from status of soil, vegetation and soil seed bank. Not applicable (-).

| Parameter                          | Renosterveld | Pine plantation | Abandoned field |
|------------------------------------|--------------|-----------------|-----------------|
| Ecosystem health <sup>#</sup>      | High         | Medium          | Low             |
| Conservation priority              | Very high    | -               | -               |
| Restoration potential and priority | -            | High            | Low             |

The genetic study with a novel multi-species and multi-fragmentation degree approach (chapter six, seven and eight, this thesis) revealed no fragmentation effects on genetic variation in *Hemimeris racemosa* and *Nemesia barbata*. No modified genetic variation has been found that might have been triggered by habitat fragmentation. Concerning fragmentation degree, no lower genetic variation within Swartland populations, a region suffering from severe fragmentation, was detected. Results suggest the presence of sufficient gene flow for the species in the past, hence no effect of fragmentation is visible yet. In order to estimate recent gene flow, more research on genetic variation of the soil seed bank and pollination and dispersal vectors is required. Renosterveld fragments offer high potential for future population genetics studies due to their interesting setting of fragments.

Additionally, the first web-based database for the Fynbos Biome was established in order to compile and make available knowledge and research about renosterveld and fynbos. Web-based content management systems, such as wikis, are an upcoming trend for information handling. This internet-based and collaborative science approach will enhance knowledge transfer and scientific progress, making literature known and connecting researchers and projects. Despite its various advantages, such as using community intelligence and preventing data loss, so far it is sparsely used within scientific communities. Together with Cornelia B. Krug (University of Cape Town), the first ever wiki for research, information and data related to the Fynbos Biome was established – *FynbosWiki* \*.

\* [http://www0.sun.ac.za/fynboswiki/index.php/Main\\_Page](http://www0.sun.ac.za/fynboswiki/index.php/Main_Page)

## Acknowledgements

I am grateful to many people, for direct and indirect help, in working at this project. I would like to express my thanks to my *Doktorvater* Peter Poschlod for his enthusiastic and expert guidance. His work and encyclopedic knowledge was very inspiring to me. I am very grateful to my other supervisors as well. I would like to thank Christoph Reisch for the DNA code of practice and many motivating words, Karen J. Esler for the warm welcome and support in South Africa and Cornelia B. Krug for advice and help that made this project running. I owe a great deal to colleagues, student friends, my girlfriend and family, who through their comments and questions have encouraged, supported and enlightened me. I have learnt a great deal from those who have worked within the project over the years and gratefully acknowledge my debt to them, especially, Sara Wennige, Barbara Hinterschwepfinger, Verena Busch, Raphael Kongor and Anne Horn (ch. 2, 3); Gyan Sharma, Arne Saatkamp, Simone Tausch and Barbara Hinterschwepfinger (ch. 4); Tygerberg Bird Club, Mooiplaas Winery, Jaques Kuyler, Kiewiett, Jackie Swart, Abraham Saaiman, Rex van Zanten, Mia Cerfonteyn and Martina Treurnicht (ch. 5); Corinna Burkhart, Philipp Glaab, Verena Busch and Franziska Kaulfuß (ch. 6-8). I am particularly grateful to Michaela Hanke, Tim Little and Maik Barthelheimer for their thoughtful and creative comments. I would like to thank the following bodies for their invaluable support, advices and sampling permits: Western Cape Nature Conservation, Tygerberg Nature Reserve, City of Cape Town, South African National Botanical Institute SANBI, CapeNature, Western Cape Nature Conservation, Friends of the Tygerberg and numerous local farmers. The technical support of Petra Schitko, Volker Debus and greenhouse staff, Wioletta Moggert and Heike Pfaffenzeller made this project running in Germany. In South Africa the thesis could only be realized with advices or technical assistance from Monean Heynes, Jaques Kuyler, Hestelle Melville and the T.N.R. staff, Jurg Zimmermann, Anna Haukka, Anne Sandbrink, Shannon Kordom, Richard Knight, Rex van Zanten, Abraham, Martina Treurnicht, Jackie Swart, Kiewiet, Rupert Koopmann, Tilla, Amrei von Hase, Antony Rebelo, Patricia Holmes, Marius Kieck, Shela Patrickson, Wendy Paisley, Karen Marais, Ruth Parker, Penny and Heidi Summer. Finally, I must acknowledge those who supported me with accommodation, such as Ulf, Marlen, Marius, Nuria, Ester and Remo. My apologies if I have inadvertently forgotten anyone to whom acknowledgement is due. While I believe that all of those mentioned have contributed to improve the thesis, none is, of course responsible for the remaining weaknesses. The project was funded by the German Federal Ministry of Education and Research (promotion number 01LC0624C).

*This thesis is dedicated to all colleagues working and enjoying renosterveld vegetation.*

## References

- Acocks, J. P. H. 1953. Veld types of South Africa. *Memoirs Botanical Survey South Africa* **28**:1-192.
- Aguiar, M. R. and O. E. Sala. 1997. Seed distribution constrains the dynamics of the Patagonian steppe. *Ecology* **78**:93-100.
- Aide, T. M. and J. Cavelier. 1994. Barriers to lowland tropical forest restoration in the Sierra Nevada de Santa Marta, Colombia. *Restoration Ecology* **2**:219-229.
- Aide, T. M., J. K. Zimmerman, L. Herrera, M. Rosario, and M. Serrano. 1995. Forest recovery in abandoned tropical pastures in Puerto Rico. *Forest Ecology and Management* **77**:77-86.
- Allcock, K. G. 2002. Effects of phosphorus on growth and competitive interactions of native and introduced species found in White Box woodlands. *Austral Ecology* **27**:638-646.
- Allsopp, N. and W. D. Stock. 1992. Density dependent interactions between VA mycorrhizal fungi and even-aged seedlings of two perennial Fabaceae species. *Oecologia* **91**:281-287.
- Allsopp, N. and W. D. Stock. 1994. VA mycorrhizal infection in relation to edaphic characteristics and disturbance regime in three lowland plant communities in the south-western Cape, South Africa. *Journal of Ecology* **82**:271-279.
- Amler, K., A. Bahl, K. Henle, G. Kaule, P. Poschlod, and J. Settele. 1999. *Populationsbiologie in der Naturschutzpraxis. Isolation, Flächenbedarf und Biotopansprüche von Pflanzen und Tieren.* Ulmer, Stuttgart, Germany.
- Archibald, S. A., D. P. Roy, B. W. Van Wilgen, and R. J. Scholes. 2009. What limits fire? An examination of drivers of burnt area in Southern Africa. *Global Change Biology* **2008**:1-18.
- Aronson, J., J. N. Blignaut, S. Milton, D. Le Maitre, K. J. Esler, A. Limouzin, C. Fontaine, M. P. de Wit, M. W., P. Prinsloo, L. van der Elst, and N. Lederer. 2010. Are Socioeconomic Benefits of Restoration Adequately Quantified? A Meta-analysis of Recent Papers (2000-2008) in Restoration Ecology and 12 Other Scientific Journals. *Restoration Ecology* **18**:143-154.
- Aronson, J., J. N. Blignaut, S. J. Milton, and A. F. Clewell. 2006a. Natural capital: The limiting factor. *Ecological Engineering* **28**:1-5.
- Aronson, J., A. F. Clewell, J. N. Blignaut, and S. J. Milton. 2006b. Ecological restoration: A new frontier for nature conservation and economics. *Journal for Nature Conservation* **14**:135-139.
- Baard, E. H. W. 1993. Distribution and status of the geometric tortoise, *Psammobates geometricus*, in South Africa. *Biological Conservation* **63**:235-239.
- Baba, W. 2004. The species composition and dynamics in well-preserved and restored calcareous xerothermic grasslands (South Poland). *Biologia* **59**:447-456.
- Bakker, J. P. and F. Berendse. 1999. Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology & Evolution* **14**:63-68.
- Bakker, J. P., P. Poschlod, R. J. Strykstra, R. M. Bekker, and K. Thomson. 1996. Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica* **45**:461-490.
- Balasse, M., S. H. Ambrose, A. B. Smith, and T. D. Price. 2002. The seasonal mobility model for prehistoric herders in the south-western Cape of South Africa assessed by isotopic analysis of sheep tooth enamel. *Journal of Archaeological Science* **29**:917-932.
- Barbaro, L., T. Dutoit, and P. Cozic. 2001. A six-year experimental restoration of biodiversity by shrub-clearing and grazing in calcareous grasslands of the French Prealps. *Biodiversity and Conservation* **10**:119-135.
- Baskin, C. and J. Baskin. 1989. Physiology of dormancy and germination in relation to seed bank ecology. Pages 53-66 in M. A. Leck, V. T. Parker, and R. L. Simpson, editors. *Ecology of soil seed banks.* Academic Press, San Diego
- Baskin, C. and J. Baskin. 1998. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination.* Academic Press, San Diego.
- Bassett, I. E., R. C. Simcock, and N. D. Mitchell. 2005. Consequences of soil compaction for seedling establishment: Implications for natural regeneration and restoration. *Austral Ecology* **30**:827-833.
- Bates, J. 2002. The genetic effects of forest fragmentation on five species of Amazonian birds. *Journal of Avian Biology* **33**:276-294.

- Baxter, B. J. M., J. Van Staden, J. E. Granger, and N. A. C. Brown. 1994. Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra*. *Environmental and Experimental Botany* **34**:217-223.
- Beatty, S. W. 1991. Colonization dynamics in a mosaic landscape: the buried seed pool. *Journal of Biogeography* **18**:553-563.
- Bijlsma, R. and V. Loeschcke. 2005. Environmental stress, adaptation and evolution: an overview. *Journal of Evolutionary Biology* **18**:744-749.
- Bishop, J. A. 1972. An experimental study of the cline of industrial melanism in *Biston betularia* (L.) (Lepidoptera) between urban Liverpool and rural North Wales. *The Journal of Animal Ecology* **41**:209-243.
- Bisteau, E., G. Mahy, and M. Pärtel. 2005. Vegetation and seed bank in a calcareous grassland restored from a *Pinus* forest. *Applied Vegetation Science* **8**:167-174.
- Blanckenhagen, B. and P. Poschlod. 2005. Restoration of calcareous grasslands: the role of the soil seed bank and seed dispersal for recolonisation processes. *Biotechnology, Agronomy, Society and Environment* **9**:143-149.
- Bond, W. J., J. Midgley, and J. Vlok. 1988. When is an island not an island? Insular effects and their causes in fynbos shrublands. *Oecologia* **77**:515-521.
- Bond, W. J., F. I. Woodward, and G. F. Midgley. 2005. Global distribution of ecosystems in a world without fire. *New Phytologist* **165**:525-538.
- Bonser, S. P. and R. J. Reader. 1995. Plant competition and herbivory in relation to vegetation biomass. *Ecology* **76**:2176-2183.
- Botha, M. 2001. Incentives for conservation on private land: options and opportunities. Cape Conservation Unit, Botanical Society of South Africa, Kirstenbosch, SA.
- Botha, M. S., P. J. Carrick, and N. Allsopp. 2008. Capturing lessons from land-users to aid the development of ecological restoration guidelines for lowland Namaqualand. *Biological Conservation* **141**:885-895.
- Boucher, C. 1980. Notes on the use of the term "Renosterveld". *Bothalia* **13**:237.
- Boucher, C. 1981. Western Cape provincial lowland alien vegetation. Pages 19–20 in *Proceedings of a Symposium on Coastal Lowlands of the Western Cape*. University of Western Cape, South Africa, Bellville.
- Boucher, C. 1983. Floristic and structural features of the coastal foreland vegetation south of the Berg River, Western Cape Province, South Africa. *Bothalia* **14**:669-674.
- Boucher, C. 1984. Patterns of invasion by larger woody alien species in selected areas of the Fynbos Biome. Pages 12–13 in *Medecos 4. Proceedings of the 4th International Conference on Mediterranean Ecosystems*. Nedlands, Botany Department, University of Western Australia, Perth.
- Boucher, C. 1987. A phytosociological study of transects through the western Cape Coastal Foreland, South Africa. PhD thesis. University of Stellenbosch, Stellenbosch.
- Boucher, C. 1995. Overview on management of renosterveld. Pages 47-50 in A. B. Low and F. E. Jones, editors. *The sustainable use and management of renosterveld remnants in the Cape Floristic Region*. Proceedings of a Symposium. FCC 1995/4. Flora Conservation Committee. Botanical Society of South Africa, Kirstenbosch, Cape Town.
- Boucher, C. and E. J. Moll. 1981. South African mediterranean shrublands. Pages 233-248 in F. di Castri, D. W. Goodall, and R. L. Specht, editors. *Mediterranean-type Shrublands*. Elsevier, Amsterdam.
- Bradshaw, A. D. 1987. Restoration: An acid test for ecology. Pages 23-30 in W. R. Jordan, M. E. Gilpin, and J. D. Aber, editors. *Restoration ecology: A synthetic approach to ecological research*. Cambridge University Press, Cambridge.
- Bradstock, R. and T. Auld. 1995. Soil temperatures during experimental bushfires in relation to fire intensity: consequences for legume germination and fire management in south-eastern Australia. *Journal of Applied Ecology* **32**:76-84.
- Bragg, C. J., J. D. Donaldson, and P. G. Ryan. 2005. Density of Cape porcupines in a semi-arid environment and their impact on soil turnover and related ecosystem processes. *Journal of Arid Environments* **61**:261-275.
- Branch, W. R. 1988. *South African Red Data Book - Reptiles and Amphibians*. CSIR, Pretoria.

- Britton, P. and J. Jackelman. 1995. Biological management of renosterveld: some questions and lessons from Signal Hill and Devil's Peak. Pages 58-62 in A. B. Low and F. E. Jones, editors. The sustainable use and management of renosterveld remnants in the Cape Floristic Region. Proceedings of a Symposium. FCC 1995/4. Flora Conservation Committee. Botanical Society of South Africa, Kirstenbosch, Cape Town.
- Brown, A. H. F. and L. Oosterhuist. 1981. The role of buried seed in coppicewoods. *Biological Conservation* **21**:19-38.
- Brown, N. A. C. 1993. Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123**:575-583.
- Brown, N. A. C. and P. A. Botha. 2004. Smoke seed germination studies and a guide to seed propagation of plants from the major families of the Cape Floristic Region, South Africa. *South African Journal of Botany* **70**:559-581.
- Brown, N. A. C., P. A. Botha, and G. Kotze. 1993. The promotion of seed germination of Cape *Erica* species by plant-derived smoke. *Seed Science and Technology* **21**:573-580.
- Bruna, E. M. and W. J. Kress. 2002. Habitat fragmentation and the demographic structure of an Amazonian understory herb (*Heliconia acuminata*). *Conservation Biology* **16**:1256-1266.
- Buisson, E., T. Dutoit, F. Torre, C. Römermann, and P. Poschlod. 2006. The implications of seed rain and seed bank patterns for plant succession at the edges of abandoned fields in Mediterranean landscapes. *Agriculture, Ecosystems and Environment* **115**:6-14.
- Bullock, J., K. Shea, and O. Skarpaas. 2006. Measuring plant dispersal: an introduction to field methods and experimental design. *Plant Ecology* **186**:217-234.
- Burchell, W. J. 1811. *Travels in the interior of Southern Africa*. Oxford University Press, London.
- Bylebyl, K. 2007. Central European dry grasslands: processes of their development and possibilities for their maintenance. *Dissertationes Botanicae*, 406. J. Cramer in der Gebr. Borntraeger Verlagsbuchhandlung, Berlin, Stuttgart.
- C.A.P.E. 2000. Renosterveld: Hidden jewels of the Cape. Botanical Society of South Africa pamphlet.
- Caldwell, M. M., D. M. Eissenstat, J. H. Richards, and M. F. Allen. 1985. Competition for Phosphorus: Differential Uptake from Dual-Isotope-Labeled Soil Interspaces Between Shrub and Grass. *Science* **229**:384-386.
- Caldwell, M. M., J. H. Richards, J. H. Manwaring, and D. M. Eissenstat. 1987. Rapid shifts in phosphate acquisition show direct competition between neighbouring plants. *Nature* **327**:615-616.
- CapeNature. 2000. Renosterveld: Landowners play a vital role in the conservation of renosterveld. CapeNature pamphlet.
- CapeNature. 2006. Protocols for restoring lowland sand fynbos. CapeNature pamphlet.
- Cilliers, C. D., K. Esler, and C. Boucher. 2004. Effects of alien plant management and fire on soil seed banks and regeneration in the Cape Peninsula National Park, South Africa. *South African Journal of Botany* **70**:705-712.
- Cione, N., P. Padgett, and E. Allen. 2002. Restoration of a native shrubland impacted by exotic grasses, frequent fire, and nitrogen deposition in southern California. *Restoration Ecology* **10**:376-384.
- Cole, C. T. 2003. Genetic variation in rare and common plants. *Annual Review of Ecology, Evolution, and Systematics* **34**:213-237.
- Connell, J. H. and R. O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *The American Naturalist* **111**:1119-1144.
- Cowling, R. M. 1984. A syntaxonomic and synecological study in the Humansdorp region of the Fynbos Biome. *Bothalia* **15**:175-227.
- Cowling, R. M. 1992. *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town.
- Cowling, R. M. and W. J. Bond. 1991. How small can reserves be? An empirical approach in Cape Fynbos, South Africa. *Biological Conservation* **58**:243-256.
- Cowling, R. M. and C. E. Heijnis. 2001. The identification of Broad Habitat Units as biodiversity entities for systematic conservation planning in the Cape Floristic Region. *South African Journal of Botany* **67**:16-38.

- Cowling, R. M., P. M. Holmes, and A. G. Rebelo. 1992. Plant diversity and endemism. Pages 62-112 in R. M. Cowling, editor. *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town.
- Cowling, R. M., D. Kirkwood, J. J. Midgley, and S. M. Pierce. 1997a. Invasion and persistence of bird-dispersed, subtropical thicket and forest species in fire-prone coastal fynbos. *Journal of Vegetation Science* **8**:475-488.
- Cowling, R. M., A. T. Knight, S. D. J. Privett, and G. P. Sharma. 2010. Invest in Opportunity, Not Inventory of Hotspots. *Conservation Biology* **24**:633-635.
- Cowling, R. M. and S. M. Pierce. 1999. Cape Floristic Province. Pages 218-227 in R. A. Mittermeier, N. Myers, and C. G. Mittermeier, editors. *Hotspots. Earth's biologically richest and threatened terrestrial ecoregions*. CEMEX Mexico City and Conservation International, Washington DC.
- Cowling, R. M., S. M. Pierce, and E. J. Moll. 1986. Conservation and utilisation of South Coast renosterveld, an endangered South African vegetation type. *Biological Conservation* **37**:363-377.
- Cowling, R. M., L. R. Pressey, A. T. Lombard, P. G. Desmet, and A. G. Ellis. 1999a. From representation to persistence: requirements for a sustainable system of conservation areas in the species-rich mediterranean-desert of Southern Africa. *Diversity and Distributions* **5**:51-71.
- Cowling, R. M. and R. L. Pressey. 2001. Rapid plant diversification: planning for an evolutionary future. *PNAS* **98**:5452-5457.
- Cowling, R. M., R. L. Pressey, A. T. Lombard, C. J. Heijnis, D. M. Richardson, and N. Cole. 1999b. Framework for a Conservation Plan for the Cape Floristic Region. Institute of Plant Conservation, Cape Town.
- Cowling, R. M., Proche, and T. C. Partridge. 2009. Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. *Molecular Phylogenetics and Evolution* **51**:64-74.
- Cowling, R. M., D. M. Richardson, P. J. Mustard, R. M. Cowling, D. M. Richardson, and S. M. Pierce. 1997b. Fynbos. Pages 99-130 in R. M. Cowling, D. M. Richardson, and S. M. Pierce, editors. *Vegetation of Southern Africa*. Cambridge University press, Cambridge.
- Cowling, R. M., P. W. Rundel, B. B. Lamont, and M. K. Arroyo. 1996. Plant diversity in mediterranean-climate regions. *Trends in Ecology and Evolution* **11**:362-366.
- Cowling, S. 2001. Lowland fynbos and renosterveld. WWF Full Report. [www.worldwildlife.org](http://www.worldwildlife.org).
- Cramer, V. A., R. J. Hobbs, and R. J. Standish. 2007. What's new about old fields? Land abandonment and ecosystem assembly. *Trends in Ecology and Evolution* **23**:104-112.
- Cramer, V. A., R. J. Hobbs, and R. J. Standish. 2008. What's new about old fields? Land abandonment and ecosystem assembly. *Trends in Ecology and Evolution* **23**:104-112.
- Crosti, R., P. G. Ladd, K. W. Dixon, and B. Piotto. 2006. Post-fire germination: the effect of smoke on seeds of selected species from the central Mediterranean basin. *Forest Ecology and Management* **221**:306-312.
- Crowe, T. M. 1990. A quantitative analysis of patterns of distribution, species richness and endemism in southern African vertebrates. Pages 124-138 in G. Peters and R. Hutterer, editors. *Vertebrates in the tropics*. Museum Alexander Koenig, Bonn.
- D'Antonio, C. and L. Meyerson. 2002. Exotic plant species as problems and solutions in ecological restoration: a synthesis. *Restoration Ecology* **10**:703-713.
- Datson, P. M., B. G. Murray, and K. R. W. Hammett. 2006. Pollination systems, hybridization barriers and meiotic chromosome behaviour in *Nemesia* hybrids. *Euphytica* **151**:173-185.
- Davies, R. J. 1985. The importance of weed control and the use of tree shelters for establishing broadleaved trees on grass-dominated sites in England. *Forestry* **58**:167-180.
- Davis, S. D., V. H. Heywood, A. C. Hamilton, S. D. Davis, V. H. Heywood, and A. C. Hamilton. 1994. Centres of plant diversity. A guide and strategy for their conservation - Europe, Africa, South West Asia and the Middle East. The World Wide Fund for Nature (WWF) and IUCN - The World Conservation Union Cambridge.
- Daws, M. I., H. W. Pritchard, and J. Van Staden. 2008. Butenolide from plant-derived smoke functions as a strigolactone analogue: Evidence from parasitic weed seed germination. *South African Journal of Botany* **74**:116-120.

- De Lange, J. H. and C. Boucher. 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany* **56**:700-703.
- de Merxem, D. G., B. Borremans, M. L. de Jäger, T. Johnson, M. Jooste, P. Ros, R. D. Zenni, A. G. Ellis, and B. Anderson. 2009. The importance of flower visitors not predicted by floral syndromes. *South African Journal of Botany* **75**:660-667.
- De Villiers, C. C., A. Driver, B. Clark, D. I. W. Euston-Brown, E. G. Day, N. Job, N. A. Helme, P. M. Holmes, S. Brownlie, and A. B. Rebelo. 2005. Fynbos Forum ecosystem guidelines for environmental assessment in the Western Cape. Fynbos Forum. Botanical Society of South Africa: Conservation Unit, Kistenbosch, Cape Town.
- Deacon, H. J., M. R. Jury, F. Ellis, and R. M. Cowling. 1992. Selective regime and time. Pages 6-22 in R. M. Cowling, editor. *The Ecology of Fynbos. Nutrients, Fire and Diversity*. Oxford University Press
- Debinski, D. M. and R. D. Holt. 2000. A survey and overview of habitat fragmentation experiments. *Conservation Biology* **14**:342-355.
- Debussche, M., J. Escarré, and J. Lepart. 1982. Ornithochory and plant succession in mediterranean abandoned orchards. *Plant Ecology* **48**:255-266.
- Debussche, M. and P. Isenmann. 1994. Bird-dispersed seed rain and seedling establishment in patchy Mediterranean vegetation. *Oikos* **69**:414-426.
- Debussche, M. and J. Lepart. 1992. Establishment of woody plants in Mediterranean old fields: opportunity in space and time. *Landscape Ecology* **6**:133-145.
- Delius, P. and S. Schirmer. 2000. Soil conservation in a racially ordered society: South Africa 1930-1970. *Journal of South African Studies* **29**:719-742.
- Di Castri, F. and H. Mooney. 1973. *Mediterranean Type Ecosystems: Origin and Structure*. Springer-Verlag, Berlin, Heidelberg, New York.
- Dietrich, K., C. Fiske, K. McAlister, E. Schobe, I. Silverman, and A. Waldron. 2004. To raise a toast: Grain and grape in the Swartland, South Africa - trends, causes and implications of agricultural land use change. Department of Environmental & Geographical Sciences at the University of Cape Town.
- Dixon, K., D. Merritt, G. Flematti, and E. Ghisalberti. 2009. Karrikinolide-a phytoactive compound derived from smoke with applications in horticulture, ecological restoration and agriculture. *Acta Horticulturae* **813**:155-170.
- Donaldson, J. S. 2002. Pollination in agricultural landscapes, a South African perspective. Pages 97-104 in P. Kevan and V. L. Imperatriz-Fonseca, editors. *Pollinating Bees - The Conservation Link Between Agriculture and Nature* Brazilian Ministry of Environment, Brasilia.
- Donaldson, J. S., I. Nänni, C. Zachariades, and J. Kemper. 2002. Effects of Habitat Fragmentation on Pollinator Diversity and Plant Reproductive Success in Renosterveld Shrublands of South Africa. *Conservation Biology* **16**:1267-1276.
- Duckitt, J. 1995. Conservation of west coast vegetation by farmers. Pages 43-46 in A. B. Low and F. E. Jones, editors. *The sustainable use and management of renosterveld remnants in the Cape Floristic Region*. Proceedings of a Symposium. FCC 1995/4. Flora Conservation Committee. Botanical Society of South Africa, Kirstenbosch, Cape Town.
- Dzwonko, Z. and S. Loster. 2007. A functional analysis of vegetation dynamics in abandoned and restored limestone grasslands. *Journal of Vegetation Science* **18**:203-212.
- Ehrlén, J. and O. Eriksson. 2003. Large-scale spatial dynamics of plants: a response to Freckleton & Watkinson. *Journal of Ecology* **91**:316-320.
- Ehrlich, P. and A. Ehrlich. 1981. *Extinction*. Random House, New York.
- Eliason, S. A. and E. B. Allen. 1997. Exotic Grass Competition in Suppressing Native Shrubland Re-establishment. *Restoration Ecology* **5**:245-255.
- Ellenberg, H. 1986. *Vegetation Mitteleuropas mit den Alpen*. Verlag Eugen Ulmer, Stuttgart.
- Ellstrand, N. C. and D. R. Elam. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* **24**:217-242.
- Ellstrand, N. C. and D. L. Marshall. 1985. Interpopulation gene flow by pollen in wild radish, *Raphanus sativus*. *The American Naturalist* **126**:606-616.
- Emery, S. M. and K. L. Gross. 2006. Dominant species identity regulates invasibility of old-field plant communities. *Oikos* **115**:549-558.

- Enright, N., D. Goldblum, P. Ata, and D. Ashton. 1997. The independent effects of heat, smoke and ash on emergence of seedlings from the soil seed bank of a heathy *Eucalyptus* woodland in Grampians (Gariwerd) National Park, western Victoria. *Austral Ecology* **22**:81-88.
- Epperson, B. 1990. Spatial autocorrelation of genotypes under directional selection. *Genetics* **124**:757-771.
- Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**:2611-2620.
- Ewel, J. J. 1999. Natural systems as models for the design of sustainable systems of land use. *Agroforestry Systems* **45**:1-21.
- Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**:479-491.
- Fahrig, L. 2001. How much habitat is enough? *Biological Conservation* **100**:65-74.
- Fairbanks, D. H. K., C. J. Hughes, and J. K. Turpie. 2004. Potential impact of viticulture expansion on habitat types in the Cape Floristic Region, South Africa. *Biodiversity and Conservation* **13**:1075-1100.
- Falk, D. A. 1990. Integrated strategies for conserving plant genetic diversity. *Annals of the Missouri Botanical Garden* **77**:38-47.
- Ferguson, B. G. 1995. Overcoming barriers to forest regeneration in a degraded tropical pasture: an evaluation of restoration techniques. MSc thesis. University of Michigan, Ann Arbor.
- Fischer, M. and D. Matthies. 1998. Effects of population size on performance in the rare plant *Gentianella germanica*. *Journal of Ecology* **86**:195-204.
- Flematti, G. R., E. L. Ghisalberti, K. W. Dixon, and R. D. Trengove. 2004. A compound from smoke that promotes seed germination. *Science* **305**:977.
- Foré, S. A., R. J. Hickey, J. L. Vankat, S. I. Guttman, and R. L. Schaefer. 1992. Genetic structure after forest fragmentation: a landscape ecology perspective on *Acer saccharum*. *Canadian Journal of Botany* **70**:1659-1668.
- Fourie, S. 2008. Composition of the soil seed bank in alien-invaded grassy fynbos: Potential for recovery after clearing. *South African Journal of Botany* **74**:445-453.
- Franklin, A. B., B. R. Noon, and T. L. George. 2002. What is habitat fragmentation? *Studies in Avian Biology* **25**:20-29.
- Frazee, S. R., R. M. Cowling, R. L. Pressey, J. K. Turpie, and N. Lindenberg. 2003. Estimating the costs of conserving a biodiversity hotspot: a case-study of the Cape Floristic Region, South Africa. *Biological Conservation* **112**:275-290.
- Gaston, K. J., T. M. Blackburn, J. J. D. Greenwood, R. D. Gregory, R. M. Quinn, and J. H. Lawton. 2000. Abundance–occupancy relationships. *Journal of Applied Ecology* **37**:39-59.
- Gaudeul, M., P. Taberlet, and I. Till-Bottraud. 2000. Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L.(Apiaceae), inferred from amplified fragment length polymorphism markers. *Molecular Ecology* **9**:1625-1637.
- Geldenhuys, C. J. 1997. Native forest regeneration in pine and eucalypt plantations in Northern Province, South Africa. *Forest Ecology and Management* **99**:101-115.
- Geldenhuys, C. J. 2002. Tropical secondary forest management in Africa: South Africa. Pages 1-40 in *Workshop on tropical secondary forest management in Africa*. FAO, Nairobi.
- Gibson, C. W. D. and V. K. Brown. 1991. The nature and rate of development of calcareous grassland in southern Britain. *Biological Conservation* **58**:297-316.
- Giladi, I. 2006. Choosing benefits or partners: a review of the evidence for the evolution of myrmecochory. *Oikos* **112**:481 - 492.
- Gitzendanner, M. A. and P. S. Soltis. 2000. Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* **87**:783-792.
- Goldblatt, P. 1997. Floristic diversity in the Cape flora of South Africa. *Biodiversity and Conservation* **6**:359-377.
- Goldblatt, P. and J. Manning. 2000. Cape plants: a conspectus of the Cape flora of South Africa. National Botanical Institute and Missouri Botanical Garden, Pretoria, SA and St. Louis, MO.
- Goldblatt, P., J. C. Manning, and D. Snijman. 2008. Cape plants: corrections and additions to the flora. 1. *Bothalia* **35**:35-46.

- Good, R. 1974. The geography of the flowering plants. Longman Group Ltd., London.
- Gosper, C. R., C. D. Stansbury, and G. Vivian-Smith. 2005. Seed dispersal of fleshy-fruited invasive plants by birds: contributing factors and management options. *Diversity and Distributions* **11**:549-558.
- Gough, M. W. and R. H. Marrs. 1990. A comparison of soil fertility between semi-natural and agricultural plant communities: implications for the creation of species-rich grassland on abandoned agricultural land. *Biological Conservation* **51**:83-96.
- Grandin, U. and H. Rydin. 1998. Attributes of the seed bank after a century of primary succession on islands in Lake Hjälmaren, Sweden. *Journal of Ecology* **86**:293-303.
- Grigulis, K., A. W. Sheppard, J. E. Ash, and R. H. Groves. 2001. The comparative demography of the pasture weed *Echium plantagineum* between its native and invaded ranges. *Journal of Applied Ecology* **38**:281-290.
- Grime, J. P. 1979. *Plant Strategies and Vegetation Processes*. Wiley, Chichester.
- Grunicke, U. 1996. Populations- und ausbreitungsbiologische Untersuchungen zur Sukzession auf Weinbergsbrachen am Keuperstufenrand des Remstales. *Dissertationes Botanicae*, 261. J. Cramer in der Gebr. Borntraeger Verlagsbuchhandlung, Berlin, Stuttgart.
- Haila, Y. 2002. A conceptual genealogy of fragmentation research: From island biogeography to landscape ecology. *Ecological Applications* **12**:321-334.
- Hall, A. V. 1981. Conservation status of the vegetation of the western Cape lowlands. Pages 57-62 in *Proceedings of a Symposium on Coastal Lowlands of the Western Cape*. University of Western Cape, South Africa, Bellville.
- Hamilton, M. B. 1994. Ex situ conservation of wild plant species: time to reassess the genetic assumptions and implications of seed banks. *Conservation Biology* **8**:39-49.
- Hamrick, J. L. and M. J. W. Godt. 1989. Allozyme diversity in plant species. Pages 43-63 in A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir, editors. *Plant Population Genetics, Breeding, and Genetic Resources*. Sinauer Associates, Sunderland, Massachusetts, USA.
- Hamrick, J. L. and M. J. W. Godt. 1996. Effects of life history traits on genetic diversity in plant species. *The Royal Society* **351**:1291-1298.
- Hamrick, J. L., Y. B. Linhart, and J. B. Mitton. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Review of Ecology and Systematics* **10**:173-200.
- Handel, S. N. 1997. The role of plant-animal mutualisms in the design and restoration of natural communities. Pages 111-132 in K. M. Urbanska, N. R. Webb, and P. J. Edwards, editors. *Restoration ecology and sustainable development*. Cambridge University Press, Cambridge.
- Hanley, M. E., M. Fenner, and G. Ne'eman. 2001. Pregermination heat shock and seedling growth of fire-following Fabaceae from four Mediterranean-climate regions. *Acta Oecologica* **22**:315-320.
- Hargrove, W. W. and J. Pickering. 1992. Pseudoreplication: a sine qua non for regional ecology. *Landscape Ecology* **6**:251-258.
- Harrison, S. and E. Bruna. 1999. Habitat fragmentation and large-scale conservation: what do we know for sure? *Ecography* **22**:225.
- Hartl, D. L. and A. G. Clark. 1989. *Principles of population genetics*. Sinauer Associates, Sunderland, Massachusetts, USA.
- Harvey, C. A. 2000. Windbreaks enhance seed dispersal into agricultural landscapes in Monteverde, Costa Rica. *Ecological Applications* **10**:155-173.
- Hedrick, P. W. and P. S. Miller. 1992. Conservation genetics: techniques and fundamentals. *Ecological Applications* **2**:30-46.
- Heelemann, S., S. Proches, S. Porembski, and R. M. Cowling. 2010. Impact of graminoid cover on post-fire growth of non-sprouting *Protea* seedlings in the eastern Fynbos Biome of South Africa. *African Journal of Ecology* **49**:51-55.
- Heelemann, S., S. Proches, A. G. Rebelo, B. W. van Wilgen, S. Porembski, and R. M. Cowling. 2008. Fire season effects on the recruitment of non-sprouting serotinous Proteaceae in the eastern (bimodal rainfall) fynbos biome, South Africa. *Austral Ecology* **33**:119-127.
- Heijnis, C. E., A. T. Lombard, R. M. Cowling, and P. G. Desmet. 1999. Picking up the pieces: a biosphere reserve framework for a fragmented landscape – The Coastal Lowlands of the Western Cape, South Africa. *Biodiversity and Conservation* **8**:471-496.

- Henderson, S., T. P. Dawson, and R. J. Whittaker. 2006. Progress in invasive plants research. *Progress in Physical Geography* **30**:25-46.
- Herranz, J. M., P. Ferrandis, and J. J. Martínez-Sánchez. 1998. Influence of heat on seed germination of seven Mediterranean *Leguminosae* species. *Plant Ecology* **136**:95-103.
- Herrera, C. M., P. Jordano, L. Lopez-Soria, and J. A. Amat. 1994. Recruitment of a mast-fruited, bird-dispersed tree: bridging frugivore activity and seedling establishment. *Ecological Monographs* **64**:315-344.
- Herrera, J. M. and D. García. 2010. Effects of Forest Fragmentation on Seed Dispersal and Seedling Establishment in Ornithochorous Trees. *Conservation Biology* **24**:1089-1098.
- Heywood, J. S. 1991. Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics* **22**:335-355.
- Hilderbrand, R. H., A. C. Watts, and A. M. Randle. 2005. The myths of restoration ecology. *Ecology and Society*: URL: <http://www.ecologyandsociety.org/vol10/iss11/art19/>.
- Hobbs, R. J., S. Arico, J. Aronson, J. S. Baron, P. Bridgewater, V. A. Cramer, P. R. Epstein, J. J. Ewel, C. A. Klink, A. E. Lugo, D. Norton, D. Ojima, D. M. Richardson, E. W. Sanderson, F. Valladares, M. Vilà, Z. Zamora, and Z. M. 2006. Novel ecosystems: theoretical and management aspects of the new ecological world order. *Global Ecology & Biogeography* **15**:1-7.
- Hobbs, R. J. and V. A. Cramer. 2007a. Old field dynamics: regional and local differences, and lessons for ecology and restoration. Pages 309-318 in V. A. Cramer and R. J. Hobbs, editors. *Old fields: Dynamics and restoration of abandoned farmland*. Island Press, Washington DC, USA.
- Hobbs, R. J. and V. A. Cramer. 2007b. Why old fields? The socioeconomic and ecological causes and consequences of land abandonment. Pages 1-14 in V. A. Cramer and R. J. Hobbs, editors. *Old fields: Dynamics and restoration of abandoned farmland*. Island Press, Washington DC, USA.
- Hobbs, R. J. and J. A. Harris. 2001. Restoration Ecology: Repairing the Earth's Ecosystems in the New Millennium. *Restoration Ecology* **9**:239-246.
- Hobbs, R. J. and L. R. Walker. 2007. Old Field Succession: Development of Concepts. Pages 17-30 in V. A. Cramer and R. J. Hobbs, editors. *Old fields: Dynamics and restoration of abandoned farmland*. Island Press, Washington DC, USA.
- Holl, K. D. 1998. Do bird perching structures elevate seed rain and seedling establishment in abandoned tropical pasture? *Restoration Ecology* **6**:253-261.
- Holl, K. D., M. E. Loik, E. H. V. Lin, and I. A. Samuels. 2000. Tropical montane forest restoration in Costa Rica: overcoming barriers to dispersal and establishment. *Restoration Ecology* **8**:339-349.
- Holmes, P. M. 2002. Depth distribution and composition of seed-banks in alien-invaded and uninvaded fynbos vegetation. *Austral Ecology* **27**:110-120.
- Holmes, P. M. 2005. Results of a lucerne old-field restoration experiment at the Fynbos-Karoo interface. *South African Journal of Botany* **71**:326 - 338.
- Holmes, P. M. 2008. Optimal ground preparation treatments for restoring lowland Sand Fynbos vegetation on old fields. *South African Journal of Botany* **74**:33-40.
- Holmes, P. M. and R. M. Cowling. 1997. Diversity, composition and guild structure relationships between soil-stored seed banks and mature vegetation in alien plant-invaded South African fynbos shrublands. *Plant Ecology* **133**:107-122.
- Holmes, P. M., S. Milton, C. B. Krug, and A. B. Rebelo. 2008. Repairing damaged habitats and restoring biodiversity. *Fynbos Management Book*.
- Holmes, P. M. and D. M. Richardson. 1999. Protocols for Restoration Based on Recruitment Dynamics, Community Structure, and Ecosystem Function: Perspectives from South African Fynbos. *Restoration Ecology* **7**:215-230.
- Holmes, P. M., D. M. Richardson, B. W. van Wilgen, and C. Gelderblom. 2000. Recovery of South African fynbos vegetation following alien woody plant clearing and fire: implications for restoration. *Austral Ecology* **25**:631-639.
- Honnay, O., D. Adriaens, E. Coart, H. Jacquemyn, and I. Roldan-Ruiz. 2007. Genetic diversity within and between remnant populations of the endangered calcareous grassland plant *Globularia bisnagarica* L. *Conservation Genetics* **8**:293-303.
- Honnay, O., B. Bossuyt, H. Jacquemyn, A. Shimono, and K. Uchiyama. 2008. Can a seed bank maintain the genetic variation in the above ground plant population? *Oikos* **117**:1-5.

- Honnay, O., E. Coart, J. Butaye, D. Adriaens, S. Van Glabeke, and I. Roldán-Ruiz. 2006. Low impact of present and historical landscape configuration on the genetics of fragmented *Anthyllis vulneraria* populations. *Biological Conservation* **127**:411-419.
- Honnay, O. and H. Jacquemyn. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology* **21**:823-831.
- Honnay, O., H. Jacquemyn, K. Van Looy, K. Vandepitte, and P. Breyne. 2009. Temporal and spatial genetic variation in a metapopulation of the annual *Erysimum cheiranthoides* on stony river banks. *Journal of Ecology* **97**:131-141.
- Hooftman, D. A. P., R. C. Billeter, B. Schmid, and M. Diemer. 2004. Genetic effects of habitat fragmentation on common species of Swiss fen meadows. *Conservation Biology* **18**:1043-1051.
- Hoppes, W. G. 1988. Seedfall pattern of several species of bird-dispersed plants in an Illinois woodland. *Ecology* **69**:320-329.
- Houghton, R. A. 1991. Tropical deforestation and atmospheric carbon dioxide. *Climatic Change* **19**:99-118.
- Huenneke, L. F., D. A. Falk, and K. E. Holsinger. 1991. Ecological implications of genetic variation in plant populations. Pages 31-44 in D. A. Falk and K. E. Holsinger, editors. *Genetics and Conservation of Rare Plants*. Oxford University Press, Oxford.
- Hulme, P. 1998. Post-dispersal seed predation: consequences for plant demography and evolution. *Perspectives in Plant Ecology, Evolution and Systematics* **1**:32-46.
- Humphrey, A. J. B. 1998. The archaeology of the Peninsula and Cape Flats. Pages 22-29 in N. M. Du Plessis, editor. *The Tygerberg - The Story of Tygerberg Hills, and the towns of Parow, Bellville and Durbanville*. Tafelberg Publishers Limited, Cape Town, South Africa.
- Humphrey, L. D. and E. W. Schupp. 2004. Competition as a barrier to establishment of a native perennial grass (*Elymus elymoides*) in alien annual grass (*Bromus tectorum*) communities. *Journal of Arid Environments* **58**:405-422.
- Hutchison, D. W. and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**:1898-1914.
- Izhaki, I., P. B. Walton, and U. N. Safriel. 1991. Seed shadows generated by frugivorous birds in an eastern mediterranean scrub. *Journal of Ecology* **79**:575-590.
- Jackson, J. 2004. The City of Cape Town's Biodiversity Network: Goals and Implementation. URL: [www.capetown.gov.za](http://www.capetown.gov.za).
- Jacquemyn, H., F. Van Rossum, R. Brys, P. Endels, M. Hermy, L. Triest, and G. De Blust. 2003. Effects of agricultural land use and fragmentation on genetics, demography and population persistence of the rare *Primula vulgaris*, and implications for conservation. *Belgian Journal of Botany* **136**:5-22.
- Janzen, D. H. 1971. Seed predation by animals. *Annual Review of Ecology and Systematics* **2**:465-492.
- Jarman, M. L. 1986. Conservation priorities in lowland regions of the fynbos biome. South African National Scientific Programmes Report No. 87, Pretoria.
- Jiménez, H. E. and J. J. Armesto. 1992. Importance of the soil seed bank of disturbed sites in Chilean matorral in early secondary succession. *Journal of Vegetation Science* **3**:579-586.
- Johnson, S. J. 1996. Pollination, adaption and speciation models in the Cape flora of South Africa. *Taxon* **45**:59-66.
- Johnson, S. J. and K. E. Steiner. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution* **15**:140-143.
- Jones, F. and K. Esler. 2004. Relationship between soil-stored seed banks and degradation in eastern Nama Karoo rangelands (South Africa). *Biodiversity and Conservation* **13**:2027-2053.
- Jordano, P. and E. W. Schupp. 2000. Seed dispersers effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecological Monographs* **70**:591-615.
- Julliot, C. 1997. Impact of seed dispersal by red howler monkeys *Alouatta seniculus* on the seedling population in the understory of tropical rain forest. *Journal of Ecology* **85**:431-440.
- Kaiser, A. 2005. Fynbos in Focus. I. Composition and potential of soil seed bank-samples from sandy proteoid Mountain-Fynbos-Communities from the Cape Peninsula, South Africa, in dependence of soil depth, fire and smoke. Diplom thesis. University of Regensburg.

- Kalabokidis, K. D., P. Konstantinidis, and C. Vasilakos. 2002. GIS analysis of physical and human impact on wildfire patterns. Pages 1-13 in Proceedings of the IV international conference on forest fire research. Forest Fire Research & Wildland Fire Safety. Millpress, Rotterdam, Netherlands, Luso, Coimbra, Portugal.
- Kalamees, R. and M. Zobel. 1998. Soil seed bank composition in different successional stages of a species rich wooded meadow in Laelatu, western Estonia. *Acta Oecologica* **19**:175-180.
- Karlík, P. and P. Poschlod. 2009. History or abiotic filter: which is more important in determining the species composition of calcareous grasslands? *Preslia* **81**:321-340.
- Keeley, J. E. and W. J. Bond. 1997. Convergent seed germination in South African fynbos and Californian chaparral. *Plant Ecology* **133**:153-167.
- Kemper, J. 1997. The effects of fragmentation of south coast renosterveld on vegetation patterns and processes. MSc thesis. University of Cape Town, Cape Town.
- Kemper, J., R. M. Cowling, and D. M. Richardson. 1999. Fragmentation of South African renosterveld shrublands: effects on plant community structure and conservation implications. *Biological Conservation* **90**:103-111.
- Kemper, J., R. M. Cowling, D. M. Richardson, G. G. Forsyth, and D. H. McKelly. 2000. Landscape fragmentation in South Coast Renosterveld, South Africa, in relation to rainfall and topography. *Austral Ecology* **25**:179-186.
- Kidd, M. M. 1983. Cape Peninsula. 5 edition. Botanical Society of South Africa, Kirstenbosch, Claremont, South Africa.
- Kiefer, S. and P. Poschlod. 1996. Restoration of fallow or afforested calcareous grasslands by clear-cutting. Pages 209-218 in J. Settele, C. Margules, P. Poschlod, and K. Henle, editors. Species survival in fragmented landscapes. Kluwer, Dordrecht.
- Klein, R. G. 1974. A provisional statement on terminal Pleistocene mammalian extinction in the Cape biotic zone (Southern Cape Province, South Africa). *South African Archaeological Society* **2**:39-45.
- Klein, R. G. 1986. The pre-history of Stone Age herders in the Cape Province of South Africa. *South African Archaeological Society Goodwin Series* **5**:5-12.
- Knight, A. T. and R. M. Cowling. 2007. Embracing Opportunism in the Selection of Priority Conservation Areas. *Conservation Biology* **21**:1124-1126.
- Knight, R. S. 1988. Aspects of plant dispersal in the southwestern Cape with particular reference to the roles of birds as dispersal agents. PhD thesis. University of Cape Town, Cape Town.
- Knight, R. S. and W. R. Siegfried. 1983. Inter-relationships between type, size and colour of fruits and dispersal in southern African trees. *Oecologia* **56**:405-412.
- Kollmann, J. 1994. Ausbreitungsökologie endozoochorer Gehölzarten. Veröffentlichung PAÖ 9, LfU Baden-Württemberg, Karlsruhe.
- Kollmann, J. and M. Pirl. 1995. Spatial pattern of seed rain of fleshy-fruited plants in a scrubland-grassland transition. *Acta Oecologica* **16**:313-329.
- Kollmann, J. and O. Wilmanns. 1994. Untersuchungen zur Entwicklung von Gehölzpflanzungen durch Neuansiedlung endozoochorer Arten in einem Rebflurbereinigungsgebiet des Kaiserstuhls. Veröffentlichung PAÖ **8**:181-195.
- Koniak, S. and R. L. Everett. 1982. Seed reserves in soils of successional stages of pinyon woodlands. *American Midland Naturalist* **108**:295-303.
- Kotze, I. 2009. Milestones and champions: news and views from the conservation unit. *Veld & Flora* **95**:3-3.
- Kovach, W. L. 1999. MVSP - A multivariate statistical Package for Windows, ver. 3.1. Kovach Computing Services, Pentraeth, Wales, UK.
- Kramer, A. T., J. L. Ison, M. V. Ashley, and H. F. Howe. 2008. The paradox of forest fragmentation genetics. *Conservation Biology* **22**:878-885.
- Kruess, A. and T. Tschardtke. 1994. Habitat fragmentation, species loss, and biological control. *Science* **264**:1581.
- Krug, C. B. 2004. Practical Guidelines for the Restoration of Renosterveld. University of Stellenbosch, Stellenbosch.
- Krug, C. B. and R. M. Krug. 2007. Restoration in old fields in renosterveld: a case study in a mediterranean-type shrubland of South Africa. Pages 265-285 in V. A. Cramer and R. J. Hobbs,

- editors. Old fields: Dynamics and restoration of abandoned farmland. Island Press, Washington DC, USA.
- Krug, C. B., R. M. Krug, D. Midoko-Iponga, B. A. Walton, S. J. Milton, and N. N. Shiponeni. 2004a. Restoration of west coast renosterveld: facilitating the return of a highly threatened vegetation type. Pages 107-119 in Proceedings of the 10th international conference on Mediterranean climate ecosystems, Rhodes, Rhodos, GR.
- Krug, R. M. 2008. Modelling seed dispersal in restoration and invasions. PhD thesis. University of Stellenbosch, Stellenbosch, SA.
- Krug, R. M., C. B. Krug, N. Farley, D. Midoko-Iponga, I. P. Newton, N. N. Shiponeni, B. A. Walton, and S. J. Milton. 2004b. Reconstructing west coast renosterveld: Past and present ecological processes in a Mediterranean shrubland of South Africa. Pages 107-119 in Proceedings of the 10th international conference on Mediterranean climate ecosystems, Rhodes, Rhodos, GR.
- Kruger, F. J. 1982. Use and management of Mediterranean Ecosystems in South Africa - Current problems. Pages 42-55 in Proceedings of the symposium on dynamics and management of Mediterranean-type ecosystems. Pacific Southwest Forest and Range Experiment Station, San Diego, California.
- Kruger, F. J. 1984. Patterns of vegetation and climate in the mediterranean zone of South Africa. Bulletin de la Société Botanique de France **131**:213-224.
- Küchler, A. W. 1967. Vegetation mapping. Ronald Press Company, New York.
- Kwak, M. and R. Bekker. 2006. Ecology of plant reproduction: extinction risks and restoration perspectives of rare plant species. Pages 362-386 in N. M. Waser and F. Ollerton, editors. Plant-pollinator interactions: from specialization to generalization. The University of Chicago Press, Chicago, London.
- Lacy, R. C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology **1**:143-158.
- Lambrechts, J. J. N. 1998. Natural resources and farming in the Tygerberg. Pages 112-126 in N. M. Du Plessis, editor. The Tygerberg: The Story of Tygerberg Hills and the towns of Parow, Bellville and Durbanville. Tafelberg, Cape Town.
- Lamont, B. B. and N. J. Enright. 2000. Adaptive advantages of aerial seed banks. Plant Species Biology **15**:157 - 166.
- Lande, R. and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. Cambridge University Press, Cambridge.
- Landergott, U., R. Holderegger, G. Kozłowski, and J. Schneller. 2001. Historical bottlenecks decrease genetic diversity in natural populations of *Dryopteris cristata*. Heredity **87**:344-355.
- Larson, A., D. B. Wake, and K. P. Yanév. 1984. Measuring gene flow among populations having high levels of genetic fragmentation. Genetics **106**:293.
- Le Maitre, D. C., B. W. van Wilgen, C. M. Gelderblom, C. Bailey, R. A. Chapman, and J. A. Nel. 2002. Invasive alien trees and water resources in South Africa: case studies of the costs and benefits of management. Forest Ecology and Management **160**:143-159.
- Lee, S. L., K. K. S. Ng, L. G. Saw, C. T. Lee, N. Muhammad, N. Tani, Y. Tsumura, and J. Koskela. 2006. Linking the gaps between conservation research and conservation management of rare dipterocarps: A case study of *Shorea lumutensis*. Biological Conservation **131**:72-92.
- Leimu, R. and P. Mutikainen. 2005. Population history, mating system, and fitness variation in a perennial herb with a fragmented distribution. Conservation Biology **19**:349-356.
- Leimu, R., P. Mutikainen, J. Koricheva, and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology **94**:942-952.
- Leishman, M. R. and V. P. Thomson. 2005. Experimental evidence for the effects of additional water, nutrients and physical disturbance on invasive plants in low fertility Hawkesbury Sandstone soils, Sydney, Australia. Journal of Ecology **93**:38-49.
- Levyns, M. R. 1935. Germination in some South African seeds. Journal of South African Botany **1**:161-170.
- Lewinton, R. C. 1972. The apportionment of human diversity. Evolutionary Biology **6**:381-398.
- Li, P. and W. T. Adams. 1989. Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). Canadian Journal of Forest Research **19**:149-161.

- Lienert, J., M. Fischer, J. Schneller, and M. Diemer. 2002. Isozyme variability of the wetland specialist *Swertia perennis* (Gentianaceae) in relation to habitat size, isolation, and plant fitness. *American Journal of Botany* **89**:801.
- Light, M. E., M. I. Daws, and J. Van Staden. 2009. Smoke-derived butenolide: towards understanding its biological effects. *South African Journal of Botany* **75**:1-7.
- Lindenmayer, D. B., J. Fischer, A. Felton, M. Crane, D. Michael, C. Macgregor, R. Montague-Drake, A. Manning, R. J. Hobbs, and C. Bradshaw. 2008. Novel ecosystems resulting from landscape transformation create dilemmas for modern conservation practice. *Conservation Letters* **1**:129-135.
- Linder, H. P. 2006. Evolution of diversity: the Cape flora. *TRENDS in Plant Science* **10**:536-541.
- Linhart, Y., J. Mitton, K. Sturgeon, and M. Davis. 1981. Genetic variation in space and time in a population of ponderosa pine. *Heredity* **46**:407-426.
- Livingston, R. B. and M. L. Alessio. 1968. Buried viable seed in successional field and forest stands, Harvard Forest, Massachusetts. *Bulletin of the Torrey Botanical Club* **95**:58-69.
- Loundou, P. M. 2008. Medicinal plant trade and opportunities for sustainable management in the Cape Peninsula, South Africa. MSc thesis. University of Stellenbosch, Stellenbosch.
- Lourens, A. C. U., A. M. Viljoen, and F. R. van Heerden. 2008. South African *Helichrysum* species: A review of the traditional uses, biological activity and phytochemistry. *Journal of Ethnopharmacology* **119**:630-652.
- Low, A. B. and F. E. Jones. 1995. The sustainable use and management of renosterveld remnants in the Cape Floristic Region. Proceedings of a Symposium. FCC 1995/4. Flora Conservation Committee. Botanical Society of South Africa, Kirstenbosch, Cape Town.
- Lugo, A. E. 1992. Comparison of tropical tree plantations with secondary forests of similar age. *Ecological Monographs* **62**:2-41.
- Luijten, S. H., A. Dierick, J. Gerard, B. Oostermeijer, L. E. L. Raijmann, and H. C. M. Den Nijs. 2000. Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in The Netherlands. *Conservation Biology* **14**:1776-1787.
- Macdonald, I. A. W. and D. M. Richardson. 1986. Alien species in terrestrial ecosystems of the fynbos biome. Pages 77-91 in I. A. W. Macdonald, F. J. Kruger, and A. A. Ferrar, editors. *The ecology and management of biological invasions in southern Africa*. Oxford University Press, Cape Town.
- Mandak, B., K. Bimova, I. Plackova, V. Mahelka, and J. Chrtek. 2005. Loss of genetic variation in geographically marginal populations of *Atriplex tatarica* (Chenopodiaceae). *Annals of Botany* **96**:901.
- Manel, S. and G. Segelbacher. 2009. Perspectives and challenges in landscape genetics. *Molecular Ecology* **18**:1821-1822.
- Manning, J. and P. Goldblatt. 1996. West Coast. Botanical Society of South Africa, Kirstenbosch, Claremont, South Africa.
- Manning, J., P. Goldblatt, and D. Snyman. 2002. *The color encyclopedia of Cape bulbs*. Timber Press, Cambridge.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**:209-220.
- Marris, E. 2009. Ragamuffin Earth. *Nature* **460**:450-453.
- Mau-Crimmins, T. 2007. Effects of removing *Cynodon dactylon* from a recently abandoned agricultural field. *Weed Research* **47**:212-221.
- McClanahan, T. R. and R. W. Wolfe. 1993. Accelerating forest succession in a fragmented landscape: the role of birds and perches. *Conservation Biology* **7**:279-288.
- McCue, K. A. and T. P. Holtsford. 1998. Seed bank influences on genetic diversity in the rare annual *Clarkia springvillensis* (Onagraceae). *American Journal of Botany* **85**:30-36.
- McCune, B. and M. J. Mefford. 1999. *Multivariate analysis on the PC-ORD system*. Version 4.2. MjM Software, Gleneden Beach, Oregon, USA.
- McDonnell, M. J. 1986. Old field vegetation height and the dispersal pattern of bird-disseminated woody plants. *Bulletin of the Torrey Botanical Club* **113**:6-11.

- McDonnell, M. J. and E. W. Stiles. 1983. The structural complexity of old field vegetation and the recruitment of bird-dispersed plant species. *Oecologia* **56**:109-116.
- McDowell, C. 1995. Grazing and renosterveld management. Pages 63-80 in A. B. Low and F. E. Jones, editors. The sustainable use and management of renosterveld remnants in the Cape Floristic Region. Proceedings of a Symposium. FCC 1995/4. Flora Conservation Committee. Botanical Society of South Africa, Kirstenbosch, Cape Town.
- McDowell, C. and E. Moll. 1992. The influence of agriculture on the decline of West Coast renosterveld, South-western Cape, South Africa. *Journal of Environmental Management* **35**:173-192.
- McKenzie, B., A. Moerat, and D. Naidoo. 1995. Traditional plant use - problems and possibilities. Pages 21-24 in A. B. Low and F. E. Jones, editors. The sustainable use and management of renosterveld remnants in the Cape Floristic Region. Proceedings of a Symposium. FCC 1995/4. Flora Conservation Committee. Botanical Society of South Africa, Kirstenbosch, Cape Town.
- Meadows, M. 2003. Soil erosion in the Swartland, Western Cape Province, South Africa: implications of past and present policy and practice. *Environmental Science and Policy* **6**:17-28.
- Memiaghe, H. R. 2008. Old field restoration: vegetation response to soil changes and restoration efforts in Western Cape Lowlands. MSc thesis. University of Stellenbosch, Stellenbosch, SA.
- Midoko-Iponga, D. 2004. Renosterveld restoration: The role of competition, herbivory and other disturbances. MSc thesis. University of Stellenbosch, Stellenbosch, South Africa.
- Midoko-Iponga, D., C. B. Krug, and S. J. Milton. 2005. Competition and herbivory influence growth and survival of shrubs on old fields: Implications for restoration of renosterveld shrubland. *Journal of Vegetation Science* **16**:658-692.
- Milberg, P. 1995. Soil seed bank after eighteen years of succession from grassland to forest. *Oikos* **72**:3-13.
- Miller, J. R. and R. J. Hobbs. 2007. Habitat Restoration - Do We Know What Were Doing? *Restoration Ecology* **15**:382-390.
- Mills, A. J., T. G. O'Connor, J. D. Bosenberg, J. Donaldson, R. G. Lechmere-Oertel, M. V. Fey, and A. Sigwela. 2003. Farming for carbon credits: implications for land use decisions in South African rangelands. *African Journal of Range and Forage Science* **20**:1540-1554.
- Milton, S. J. 2004. Grasses as invasive alien plants in South Africa. *South African Journal of Science* **100**:69-75.
- Mittermeier, R. A., N. Myers, J. B. Thomsen, G. A. B. da Fonseca, and S. Olivieri. 1998. Biodiversity hotspots and major tropical wilderness areas: approaches to setting conservation priorities. *Conservation Biology* **12**:516-520.
- Mix, C., P. F. P. Arens, R. Rengelink, M. J. M. Smulders, J. M. Van Groenendael, and N. J. Ouborg. 2006. Regional gene flow and population structure of the wind-dispersed plant species *Hypochaeris radicata* (Asteraceae) in an agricultural landscape. *Molecular Ecology* **15**:1749-1758.
- Moerat, A. 1995. Medicinal plant use by Rastafarians in the south west Cape. MSc thesis. University of the Western Cape, Bellville.
- Moll, E. J., B. M. Campbell, R. M. Cowling, L. Bossi, M. L. Jarman, and C. Boucher. 1984. Description of major vegetation categories in and adjacent to the Fynbos biome. *Council for Scientific and Industrial Research* **83**:11-14.
- Montalvo, A. M., S. L. Williams, K. J. Rice, S. L. Buchmann, C. Cory, S. N. Handel, G. P. Nabhan, R. Primack, and R. H. Robichaux. 1997. Restoration Biology: A Population Biology Perspective. *Restoration Ecology* **5**:277-290.
- Mooney, H. A. and E. E. Cleland. 2001. The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences* **98**:5446-5451.
- Mueller-Dombois, D. and H. Ellenberg. 1974. Aims and methods of vegetation ecology. John Wiley & Sons, New York.
- Muhl, S. A. 2008. Alien grass invasion of Renosterveld: Influence of soil variable gradients. MSc thesis. University of Stellenbosch, Stellenbosch.
- Muir, G., A. J. Lowe, C. C. Fleming, and C. Vogl. 2004. High nuclear genetic diversity, high levels of outcrossing and low differentiation among remnant populations of *Quercus petraea* at the margin of its range in Ireland. *Annals of Botany* **93**:691-697.

- Musil, C. F., S. J. Milton, and D. G. W. 2005. The threat of alien invasive grasses to lowland Cape floral diversity: an empirical appraisal of the effectiveness of practical control strategies. *South African Journal of Science* **101**:337-344.
- Myers, J. S. 1997. Geology of granite. *Journal of the Royal Society of Western Australia* **80**:87-100.
- Myers, N. 2001. Mediterranean-climate regions: glowing hotspots of diversity. *Journal of Mediterranean Ecology* **2**:157-163.
- Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**:853-858.
- Myster, R. W. and S. T. A. Pickett. 1990. Initial conditions, history and successional pathways in ten contrasting old fields. *American Midland Naturalist* **124**:231-238.
- Naidoo, D. 1994. Investigation of population structure of two frequently used species by herbalists. Honors thesis. University of the Western Cape, Bellville.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**:583-590.
- Nel, J. L., D. M. Richardson, M. Rouget, T. N. Mgidi, N. Mdzeke, D. C. Maitre, B. W. Wilgen, L. Schonegevel, L. Henderson, and S. Naser. 2004. A proposed classification of invasive alien plant species in South Africa: towards prioritizing species and areas for management action. *South African Journal of Science* **100**:53-64.
- Nelson, D., J. Riseborough, G. Flematti, J. Stevens, E. Ghisalberti, K. Dixon, and S. Smith. 2009. Karrikins discovered in smoke trigger *Arabidopsis* seed germination by a mechanism requiring gibberellic acid synthesis and light. *Plant physiology* **149**:863-873.
- Newton, I. P. 2008. Recent transformations in the west-coast renosterveld: Patterns, processes, and ecological significance. PhD thesis. University of the Western Cape, Bellville, RSA.
- Newton, I. P. and R. Knight. 2004. The Matrix - The evolutionary development of coastal renosterveld. *Veld & Flora* **90**:162-165.
- Newton, I. P. and R. S. Knight. 2005. The use of a 60-year series of aerial photographs to assess local agricultural transformations of West Coast Renosterveld, an endangered South African vegetation type. *South African Geographical Journal* **87**:18-27.
- Nybom, H. and V. Bartish. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics* **3**:93-114.
- O'Farrell, P. J. and S. Collard. 2003. Ecosystem services: an examination of approaches to investigating ecosystem services in agricultural landscapes in South Africa and Australia. Pages 1513-1514 in VIIth International Rangelands Congress, Durban, South Africa.
- O'Farrell, P. J., J. S. Donaldson, and M. T. Hoffman. 2009. Local benefits of retaining natural vegetation for soil retention and hydrological services. *South African Journal of Botany* **75**:573-583.
- Olmstead, R. G., C. W. de Pamphilis, A. D. Wolfe, N. D. Young, W. J. Elisons, and P. A. Reeves. 2001. Disintegration of the Scrophulariaceae. *American Journal of Botany* **88**:348-361.
- Oostermeijer, J., M. Brugman, E. De Boer, and H. Den Nijs. 1996. Temporal and spatial variation in the demography of *Gentiana pneumonanthe*, a rare perennial herb. *Journal of Ecology* **84**:153-166.
- Oostermeijer, J. G. B., S. H. Luijten, and J. C. M. Den Nijs. 2003. Integrating demographic and genetic approaches in plant conservation. *Biological Conservation* **113**:389-398.
- Oosting, H. J. and M. E. Humphreys. 1940. Buried viable seeds in a successional series of old field and forest soils. *Bulletin of the Torrey Botanical Club* **67**:253-273.
- Ouborg, N. J., P. Vergeer, and C. Mix. 2006. The rough edges of the conservation genetics paradigm for plant. *Journal of Ecology* **94**:1233-1248.
- Pärtel, M., R. Kalamees, M. Zobel, and E. Rosén. 1998. Restoration of species-rich limestone grassland communities from overgrown land: the importance of propagule availability. *Ecological Engineering* **10**:275-286.
- Pausas, J. G., A. Bonet, F. T. Maestre, and A. Climent. 2006. The role of the perch effect on the nucleation process in Mediterranean semi-arid oldfields. *Acta Oecologica* **29**:346-352.
- Pauw, A. 2004. Variation in pollination across a fragmented landscape at the Cape of Africa. PhD thesis. University of Cape Town, Cape Town, South Africa.

- Pauw, A. 2005. Inversostyly: a new stylar polymorphism in an oil-secreting plant, *Hemimeris racemosa* (Scrophulariaceae). *American Journal of Botany* **92**:1878-1886.
- Pauw, A. 2007. Collapse of a pollination web in small conservation areas. *Ecology* **88**:1759-1769.
- Pauw, A., W. J. Bond, and J. A. Hawkins. 2004. Reconstruction of a historical pollination landscape reveals the disruption of mutualism in small conservation areas. Pages "104.pdf", CD file in Proceedings of 10th MEDECOS Conference, Rhodes, Greece.
- Peakall, R. O. D. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**:288-295.
- Pérez-Fernández, M. A. and S. Rodríguez-Echeverría. 2003. Effect of smoke, charred wood, and nitrogenous compounds on seed germination of ten species from woodland in Central-Western Spain. *Journal of Chemical Ecology* **29**:237-251.
- Philogene, D. 1995. The use of renosterveld remnants for birding activities and as a recreational facility. Pages 14-18 in A. B. Low and F. E. Jones, editors. The sustainable use and management of renosterveld remnants in the Cape Floristic Region. Proceedings of a Symposium. FCC 1995/4. Flora Conservation Committee. Botanical Society of South Africa, Kirstenbosch, Cape Town.
- Pickett, S. T. A. 1989. Space-for-time substitution as an alternative to long-term studies. Springer-Verlag, New York, New York, USA.
- Pierce, S. M., K. Esler, and R. M. Cowling. 1995. Smoke-induced germination of succulents (Mesembryanthemaceae) from fire-prone and fire-free habitats in South Africa. *Oecologia* **102**:520-522.
- Piggin, C. M. 1976. Factors affecting seed germination of *Echium plantagineum* L. and *Trifolium subterraneum* L. *Weed Research* **16**:337-344.
- Piggin, C. M. 1978. Flowering and seed production of *Echium plantagineum* L. *Weed Research* **18**:83-87.
- Piqueray, J., G. Bottin, L. Delescaille, E. Bisteau, G. Colinet, and G. Mahy. 2010. Rapid restoration of a species-rich ecosystem assessed from soil and vegetation indicators: the case of calcareous grasslands restored from forest stands. *Ecological Indicators* **11**:724-733.
- Poschlod, P., J. Bakker, S. Bonn, and S. Fischer. 1996. Dispersal of plants in fragmented landscapes. Pages 123-127 in J. Settele, C. Margules, P. Poschlod, and K. Henle, editors. Species Survival in Fragmented Landscapes. Kluwer Academic Publishers, Dordrecht, Boston, London.
- Poschlod, P. and S. Jordan. 1992. Recolonization of an afforested calcareous oligotrophic grassland community site after clearing. *Zeitschrift für Ökologie und Naturschutz* **1**:119-139.
- Poschlod, P., S. Kiefer, U. Tränkle, S. Fischer, and S. Bonn. 1998. Plant species richness in calcareous grasslands is effected by dispersibility in space and time. *Applied Vegetation Science* **1**:75-90.
- Powrie, F. 1995. The horticultural use of renosterveld. Pages 25-30 in A. B. Low and F. E. Jones, editors. The sustainable use and management of renosterveld remnants in the Cape Floristic Region. Proceedings of a Symposium. FCC 1995/4. Flora Conservation Committee. Botanical Society of South Africa, Kirstenbosch, Cape Town.
- Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**:945-959.
- Proches, S., R. M. Cowling, P. Goldblatt, J. C. Manning, and D. A. Snijman. 2006. An overview of the Cape geophytes. *Biological Journal of the Linnean Society* **87**:27-43.
- Pueyo, Y. and C. L. Alados. 2007. Effects on fragmentation, abiotic factors and land use on vegetation recovery in a semi-arid Mediterranean area. *Basic and Applied Ecology* **8**:158-170.
- Radloff, F. G. T. 2008. The ecology of large herbivores native to the coastal lowlands of the fynbos biome in the Western Cape, South Africa. PhD thesis. University of Stellenbosch, Stellenbosch.
- Raitt, G. R. 2005. *Themeda triandra* renosterveld in the Heidelberg district. MSc thesis. University of Stellenbosch, Stellenbosch.
- Ramankutty, N. and J. A. Foley. 1999. Estimating historical changes in global land cover: Croplands from 1700 to 1992. *Global Biogeochemical Cycles* **13**:997-1027.
- Rebello, A. B. 1995. Renosterveld: conservation and research. Pages 32-42 in A. B. Low and F. E. Jones, editors. The sustainable use and management of renosterveld remnants in the Cape Floristic Region. Proceedings of a Symposium. FCC 1995/4. Flora Conservation Committee. Botanical Society of South Africa, Kirstenbosch, Cape Town.

- Rebello, A. G. 1992a. Preservation of biotic diversity. Pages 309-344 in R. M. Cowling, editor. The Ecology of Fynbos: Nutrients, Fire and Diversity. Oxford University Press, Cape Town.
- Rebello, A. G. 1992b. Red Data Book species in the Cape Floristic Region: threats, priorities and target species. Transactions of the Royal Society of South Africa **48**:55-86.
- Rebello, A. G. 1996. West Coast Renosterveld. in A. B. Low and A. G. Rebello, editors. Vegetation of South Africa, Lesotho and Swaziland. Department of Environmental Affairs and Tourism, Pretoria.
- Rebello, A. G. 1997. Conservation. Pages 571-590 in R. M. Cowling, D. M. Richardson, and S. M. Pierce, editors. Vegetation of Southern Africa. Cambridge University press, Cambridge.
- Rebello, A. G., C. Boucher, N. Helme, L. Mucina, M. C. Rutherford, W. J. Smit, L. W. Powrie, F. Ellis, J. J. Lambrechts, and L. Scott. 2006. Fynbos biome. Pages 53-219 in L. Mucina and M. C. Rutherford, editors. The Vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria, South Africa. Strelitzia.
- Reisch, C. 2008. Glacial history of *Saxifraga paniculata* (Saxifragaceae): molecular biogeography of a disjunct arctic-alpine species from Europe and North America. Biological Journal of the Linnean Society **93**:385-398.
- Reisch, C., A. Anke, and M. Röhl. 2005. Molecular variation within and between ten populations of *Primula farinosa* (Primulaceae) along an altitudinal gradient in the northern Alps. Basic and Applied Ecology **6**:35-45.
- Reisch, C., A. J. Kaiser, A. Horn, and P. Poschlod. 2010. Genetic variation of *Mimetes hirtus* and *Mimetes fimbriifolius* (Proteaceae)—a comparative analysis of two closely related fynbos species. Plant Biology **12**:537 - 544.
- Richardson, D. M., P. A. Williams, and R. J. Hobbs. 1994. Pine Invasions in the Southern Hemisphere: Determinants of Spread and Invadability. Journal of Biogeography **21**:511-527.
- Robinson, G. R. and S. N. Handel. 1993. Forest restoration on a closed landfill: rapid addition of new species by bird dispersal. Conservation Biology **7**:271-278.
- Robinson, G. R. and J. F. Quinn. 1992. Habitat fragmentation, species diversity, extinction, and design of nature reserves. Monographiae Biologicae **67**:223-248.
- Rogers, S. and A. Bendich. 1994. Extraction of total cellular DNA from plants, algae and fungi. Plant Molecular Biology Manual **1**:1-8.
- Römermann, C., T. Dutoit, P. Poschlod, and E. Buisson. 2005. Influence of former cultivation on the unique Mediterranean steppe of France and consequences for conservation management. Biological Conservation **121**:21-33.
- Rouget, M., D. M. Richardson, R. M. Cowling, J. W. Lloyd, and A. T. Lombard. 2003. Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape floristic region, South Africa. Biological Conservation **112**:63-85.
- Rutherford, M. C., G. F. Midgley, W. J. Bond, L. W. Powrie, R. Roberts, and J. Allsopp. 1999. Plant biodiversity: vulnerability and adaptation assessment. National Botanical Institute, Cape Town, South Africa.
- Ruwanza, S. 2008. Resource manipulation for control of alien invasive grasses in the Fynbos Biome. PhD thesis. University of the Western Cape, Cape Town.
- Saayman, N. and J. C. Botha. 2008. Does the soil seed bank of veld dominated by *Pteronia paniculata* change as a result of brush-cutting? Grassroots: Newsletter of the Grassland Society of Southern Africa **8**:32-35.
- Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature **392**:491-494.
- Sala, O. E., F. S. Chapin Iii, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, and A. Kinzig. 2000. Global biodiversity scenarios for the year 2100. Science **287**:1770.
- Saunders, D. A., R. J. Hobbs, and C. R. Margules. 1991. Biological Consequences of Ecosystem Fragmentation: A Review. Conservation Biology **5**:18-32.
- Schlichting, E. and H. P. Blume. 1966. Bodenkundliches Praktikum. Parey, Berlin, Hamburg.
- Schmidt, K. and K. Jensen. 2000. Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (Scrophulariaceae) and its relation to population size and reproductive components. American Journal of Botany **87**:678.

- Schmitt, J. and S. E. Gamble. 1990. The effect of distance from the parental site on offspring performance and inbreeding depression in *Impatiens capensis*: a test of the local adaptation hypothesis. *Evolution* **44**:2022-2030.
- Schweitzer, F. R. and K. J. Scott. 1973. Early occurrence of domestic sheep in sub-Saharan Africa. *Nature* **241**:547.
- Schwilk, D. W. and D. D. Ackerly. 2001. Flammability and serotiny as strategies: correlated evolution in pines *Oikos* **94**:326-336.
- Sealy, J. and R. Yates. 1994. The chronology of the introduction of pastoralism to the Cape, South Africa. *Antiquity* **68**:58-67.
- Sharma, G. P. and K. J. Esler. 2008. Phenotypic plasticity among *Echium plantagineum* populations in different habitats of Western Cape, South Africa. *South African Journal of Botany* **74**:746-749.
- Sharma, G. P., S. A. Muhl, K. J. Esler, and S. J. Milton. 2010. Competitive interactions between the alien invasive annual grass *Avena fatua* and indigenous herbaceous plants in South African Renosterveld: the role of nitrogen enrichment. *Biological Invasions* **12**:3371-3378.
- Shea, K. 2007. How the Wood Moves. *Science* **315**:1231-1232.
- Shiponeni, N. N. 2003. Dispersal of seeds as a constraint in revegetation of old fields in Renosterveld vegetation in the Western Cape, South Africa. MSc thesis. University of Stellenbosch.
- Shiponeni, N. N. and S. J. Milton. 2006. Seed dispersal in the dung of large herbivores: implications for restoration of Renosterveld shrubland old fields. *Biodiversity and Conservation* **15**:3161-3175.
- Skead, C. J. 1980. Historical mammal incidence in the Cape Province. Vol.1. The Western and Northern Cape. Department of Nature and Environmental Conservation of the Provincial Administration of the Cape of Good Hope, Cape Town, RSA.
- Skelton, P. H., J. A. Cambray, A. Lombard, and G. A. Benn. 1995. Patterns of distribution and conservation status of freshwater fishes in South Africa. *South African Journal of Zoology* **30**:71-81.
- Slatkin, M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* **16**:393-430.
- Smith, A. J. 1975. Invasion and ecesis of bird-disseminated woody plants in a temperate forest sere. *Ecology* **56**:19-34.
- Sokal, R. R. and F. J. Rohlf. 1995. *Biometry - the principles and practice of statistics in biological research*. 3 edition. Freeman, New York.
- Soltis, P. S. and M. A. Gitzendanner. 1999. Molecular Systematics and the Conservation of Rare Species. *Conservation Biology* **13**:471-483.
- Sork, V. L., J. Nason, D. R. Campbell, and J. F. Fernandez. 1999. Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology and Evolution* **14**:219-223.
- Soulé, M. E. 1985. What is conservation biology? *BioScience* **35**:727-734.
- Specht, R. L. and E. J. Moll. 1983. Mediterranean-type heathlands and sclerophyllous shrublands of the world: An overview. Pages 41-65 in F. J. Kruger, D. T. Mitchell, and J. U. M. Jarvis, editors. *Mediterranean type ecosystems. The role of nutrients*. Springer Verlag, Berlin, Heidelberg, New York, Tokyo.
- Standish, R. J., V. A. Cramer, S. b. Wild, and R. J. Hobbs. 2007. Seed dispersal and recruitment limitation are barriers to native recolonization of old-fields in western Australia. *Journal of Applied Ecology* **44**:435-445.
- Stanway, R. 2007. Alien grass infestation in renosterveld fragments: Effect on threatened life-history types and potential controls. *South African Journal of Botany* **73**:313-314.
- Stegelmeier, B., D. Gardner, and T. Davis. 2009. Livestock Poisoning With Pyrrolizidine-Alkaloid-Containing Plants (*Senecio*, *Crotalaria*, *Cynoglossum*, *Amsinckia*, *Heliotropium*, and *Echium* spp.). *Rangelands* **31**:35-37.
- Steiner, K. E. 1996. Chromosome numbers and relationships in tribe Hemimerideae (Scrophulariaceae). *Systematic Botany* **21**:63-76.
- Steiner, K. E. and V. B. Whitehead. 2002. Oil secretion and the pollination of *Colpias mollis* (Scrophulariaceae). *Plant Systematics and Evolution* **235**:53-66.
- Steubing, L. and A. Fangmeier. 1992. *Pflanzenökologisches Praktikum*. Ulmer, Stuttgart.

- Stevens, J. C., D. J. Merritt, G. R. Flematti, E. L. Ghisalberti, and K. W. Dixon. 2007. Seed germination of agricultural weeds is promoted by the butenolide 3-methyl-2H-furo[2,3-c]pyran-2-one under laboratory and field conditions. *Plant and Soil* **298**:113-124.
- Struckenberg, B. R. 1962. The distribution of the montane palaeogenic element in the South African invertebrate fauna. *Annals of the Cape Provincial Museum* **11**:119-158.
- Stylinski, K. N. and E. B. Allen. 1999. Lack of native species recovery following severe exotic disturbance in southern Californian shrublands. *Journal of Applied Ecology* **36**:544-554.
- Suding, K. N., K. D. LeJeune, and T. R. Seastedt. 2004. Competitive impacts and responses of an invasive weed: dependencies on nitrogen and phosphorus availability. *Oecologia* **141**:526-535.
- Syphard, A. D., V. C. Radeloff, J. E. Keeley, T. J. Hawbaker, M. K. Clayton, S. I. Stewart, and R. B. Hammer. 2007. Human influence on California fire regimes. *Ecological Applications* **17**:1388-1402.
- Takhtajan, A. 1969. Flowering plants - origin and dispersal. Bishen Singh Mahendra Pal Singh & Otto Koeltz Science Publishers, Dehra Dun, India; Koenigsstein, Germany
- Talbot, W. J. 1947. Swartland and sandveld. Oxford University Press, Cape Town, RSA.
- Tansley, S. A. 1988. The status of threatened Proteaceae in the Cape flora, South Africa, and the implications for their conservation. *Biological Conservation* **43**:227-239.
- Templeton, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* **7**:381-397.
- Ter Heerdt, G. N. J., G. L. Verweij, R. M. Bekker, and J. P. Bakker. 1996. An improved method for seed-bank analysis: Seedling emergence after removing the soil by sieving. *Functional Ecology* **10**:144-151.
- Theal, G. M. C. 1922. History of South Africa. Struik, Cape Town.
- Thom, H. B. 1952. Journal of Jan van Riebeck, Volume 1: 1651-1655. Van Riebeck Society, Cape Town, RSA.
- Thom, H. B. 1954. Journal of Jan van Riebeck, Volume 2: 1656-1658. Van Riebeck Society, Cape Town, RSA.
- Thompson, K. 2000. The functional ecology of soil seed banks. Pages 215-235 in M. Fenner, editor. *Seeds: the ecology of regeneration in plant communities*. Wallingford, CAB International.
- Thompson, K., J. P. Bakker, and R. M. Bekker. 1997. The soil seed banks of North West Europe: methodology, density and longevity. Cambridge University Press.
- Toerien, W. 2000. Wines and Vineyards of South Africa. Struik, Cape Town.
- Traveset, A. 1998. Effect of seed passage through vertebrate frugivores' guts on germination: a review. *Perspectives in Plant Ecology, Evolution and Systematics* **1**:151-190.
- Travis, S. E. and M. W. Hester. 2005. A space-for-time substitution reveals the long-term decline in genotypic diversity of a widespread salt marsh plant, *Spartina alterniflora*, over a span of 1500 years. *Journal of Ecology* **93**:417-430.
- Travis, S. E., J. Maschinski, and P. Keim. 1996. An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. *Molecular Ecology* **5**:735-745.
- Trinder-Smith, T. H. and M. R. Levyns. 2003. The Levyns guide to the plant genera of the Southwestern Cape. Published for Bolus Herbarium by Red Roof Design, Cape Town.
- Trollope, W. S. W. 1970. A consideration of macchia (fynbos) encroachment in South Africa and an investigation into methods of macchia eradication in the Amatole Mountains. MSc thesis. University of Natal, Pietermaritzburg.
- Turner, I. M. 1996. Species loss in fragments of tropical rain forest: a review of the evidence. *Journal of Applied Ecology* **33**:200-209.
- Underwood, E. C., J. H. Viers, K. R. Klausmeyer, R. L. Cox, and M. R. Shaw. 2009. Threats and biodiversity in the mediterranean biome. *Diversity and Distributions* **15**:188-197.
- Valentyn, F. 1726. Oud en Nieuw Oost Indien Verhandeling der ZeeHorenkens Banda. Dordrecht, Amsterdam.
- Vallejo, R., J. Aronson, J. G. Pausas, and J. Cortina. 2005. Restoration of Mediterranean woodlands. Pages 193-207 in J. Van Andel and J. Aronson, editors. *Restoration Ecology*. Blackwell Publishing.

- van Andel, J. and J. Aronson. 2006. *Restoration Ecology: The New Frontier*. Blackwell Publishing, Oxford.
- Van Assche, J. A., K. L. A. Debucquoy, and W. A. F. Rommens. 2003. Seasonal cycles in the germination capacity of buried seeds of some Leguminosae (Fabaceae). *New Phytologist* **158**:315-323.
- van Rooyen, S. 2004. Factors affecting alien grass invasion into west coast renosterveld fragments. MSc thesis. University of Stellenbosch, Stellenbosch.
- Van Rossum, F., S. C. D. Sousa, and L. Triest. 2004. Genetic consequences of habitat fragmentation in an agricultural landscape on the common *Primula veris*, and comparison with its rare congener, *P. vulgaris*. *Conservation Genetics* **5**:231-245.
- Van Staden, J., N. A. C. Brown, A. K. Jäger, and T. A. Johnson. 2000. Smoke as a germination cue. *Plant Species Biology* **15**:167-178.
- Van Staden, J., A. K. Jäger, M. E. Light, and B. V. Burger. 2004. Isolation of the major germination cue from plant-derived smoke. *South African Journal of Botany* **70**:654-659.
- van Wilgen, B. W. 2009. The evolution of fire and invasive alien plant management practices in fynbos. *South African Journal of Science* **105**:335-342.
- van Wilgen, B. W., W. J. Bond, and D. M. Richardson. 1992. Ecosystem management. Pages 345-371 in R. M. Cowling, editor. *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town.
- Verdú, M. and P. García-Fayos. 1996. Nucleation processes in a Mediterranean bird-dispersed plant. *Functional Ecology* **10**:275-280.
- von Hase, A., M. Rouget, K. Maze, and N. A. Helme. 2003a. A fine-scale conservation plan for Cape Lowlands Renosterveld. Full Report., Conservation Unit. Botanical Society of SA, Cape Town.
- von Hase, A., M. Rouget, K. Maze, and N. A. Helme. 2003b. A fine-scale conservation plan for Cape Lowlands Renosterveld. Summary Report., Conservation Unit. Botanical Society of SA, Cape Town.
- Vos, P., R. Hogers, M. Blekker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**:4407-4414.
- Vrdoljak, S. M. and M. J. Samways. 2005. A special case for the generalists? Flower visitors in the lowlands of the CFR. Entomological Society of Southern Africa Congress, Rhodes University, Grahamstown, SA.
- Wagner, M., P. Poschlod, and R. P. Setchfield. 2003. Soil seed banks in managed and abandoned semi-natural meadows in Sooma National Park, Estonia. *Annales Botanici Fennici* **40**:87-100.
- Walker, L. and R. del Moral. 2008. Transition dynamics in succession: implications for rates, trajectories and restoration. Pages 33-49 in R. J. Hobbs and K. N. Suding, editors. *New models for ecosystem dynamics and restoration*. Island Press, Washington, USA.
- Walton, B. J. 2006. Vegetation patterns and dynamics of renosterveld at Agter-Groeneberg Conservancy, Western Cape, South Africa. MSc thesis. University of Stellenbosch, Stellenbosch, South Africa.
- Waterhouse, G. 1932. Simon van der Stel's journal of his expedition to Namaqualand 1685-6. Hodges, Figgus, Dublin.
- Watson-Jones, S. J., N. Maxted, and B. V. Ford-Lloyd. 2006. Population baseline data for monitoring genetic diversity loss for 2010: a case study for *Brassica* species in the UK. *Biological Conservation* **132**:490-499.
- Wesson, A. 1998. The Tygerberg Hills 1652 to 1945. in N. M. Du Plessis, editor. *The Tygerberg - The Story of Tygerberg Hills, and the towns of Parow, Bellville and Durbanville*. Tafelberg Publishers Limited, Cape Town, South Africa.
- Whisenant, S. G. 1999. *Repairing damaged wildlands: a process-oriented, landscape-scale approach*. Cambridge University Press, Cambridge.
- Whitehead, V. and K. Steiner. 2001. Oil-collecting bees of the winter rainfall area of South Africa (Melittidae, *Rediviva*). *Annals of South African Museum* **108**.
- Willems, J. H. and L. P. M. Bik. 1998. Restoration of high species density in calcareous grassland: the role of seed rain and soil seed bank. *Applied Vegetation Science* **1**:91-100.

- Williams, P., L. Hannah, S. Andelman, G. F. Midgley, M. Araujo, G. Hughes, L. Manne, E. Martinez-Meyer, and R. Pearson. 2005. Planning for Climate Change: Identifying Minimum-Dispersal Corridors for the Cape Proteaceae. *Conservation Biology* **19**:1063-1074.
- Willson, M. F. and F. H. J. Crome. 1989. Patterns of seed rain at the edge of a tropical Queensland rainforest. *Journal of Tropical Ecology* **5**:301-308.
- Wilson, F. 1971. Farming, 1866-1966. Pages 104-153 in M. Wilson and L. Thompson, editors. *The Oxford History of South Africa II: 1870-1966*. Oxford University Press, London.
- Winter, S. J. 2003. Attitudes and behavior of landholders towards the conservation of Overberg coastal renosterveld, a threatened vegetation type in Cape floral Kingdom. MSc thesis. University of Stellenbosch, Stellenbosch, SA.
- Winter, S. J., K. J. Esler, and M. Kidd. 2005. An index to measure the conservation attitudes of landowners towards Overberg Coastal Renosterveld, a critically endangered vegetation type in the Cape Floral Kingdom, South Africa. *Biological Conservation* **126**:383-394.
- Wood, J. and A. B. Low. 1993a. Environmental survey and management guidelines for the Tygerberg and environs: Part one – Environmental Survey. National Botanical Institute, Kirstenbosch.
- Wood, J. and A. B. Low. 1993b. Environmental survey and management guidelines for the Tygerberg and environs: Part two - Management guidelines. National Botanical Institute, Kirstenbosch.
- Wood, J. and A. B. Low. 1998. The Ecology and Conservation of the Tygerberg. Pages 127-150 in N. M. Du Plessis, editor. *The Tygerberg - The Story of Tygerberg Hills, and the towns of Parow, Bellville and Durbanville*. Tafelberg Publishers Limited, Cape Town, South Africa.
- Wood, J., A. B. Low, J. S. Donaldson, and A. G. Rebelo. 1994. Threats to plant species diversity through urbanization and habitat fragmentation in the Cape Metropolitan Area, South Africa. *Strelitzia* **1**:259-274.
- Wunderle, J. 1997. The role of animal seed dispersal in accelerating native forest regeneration on degraded tropical lands. *Forest Ecology and Management* **99**:223-235.
- Yeh, F. C., R. C. Yang, and T. B. J. Boyle. 1999. POPGENE. Microsoft Windows-based Freeware for Population Genetic Analysis. Release 1.31.
- Young, A., T. Boyle, and T. Brown. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution* **11**:413-418.
- Young, A. G., A. H. D. Brown, and F. A. Zich. 1999. Genetic structure of fragmented populations of the endangered daisy *Rutidosia leptorrhynchoides*. *Conservation Biology* **13**:256-265.
- Young, A. G., H. G. Merriam, and S. I. Warwick. 1993. The effects of forest fragmentation on genetic variation in *Acer saccharum* Marsh (sugar maple) populations. *Heredity* **71**:277-289.
- Young, T. P. 2000. Restoration ecology and conservation biology. *Biological Conservation* **92**:73-83.
- Zahawi, R. A. and C. K. Augspurger. 2008. Tropical forest restoration: tree islands as recruitment foci in degraded lands of Honduras. *Ecological Applications* **16**:464-478.