

REPRODUCTIVE BIOLOGY, ECOLOGY AND HISTORIC
BIOGEOGRAPHY OF SELECTED RARE AND ENDANGERED *OXALIS*
L. (OXALIDACEAE) SPECIES

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DECLARATION

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

02/02/2007

Date

SUMMARY

Twenty five percent of all southern African *Oxalis* species are rare/endangered and highly localized, making them especially vulnerable to threats of extinction (inbreeding, low genetic variation, disrupted biological interactions) associated with small, isolated populations. In addition, *Oxalis* also displays tristylly, a restrictive breeding system that includes a strong self-incompatibility component that promotes out-crossing within populations. As it requires equal availability of three different floral morph types as well as effective pollinators for seed production, this breeding system can be a further threat to small, isolated populations when it is fully expressed. Breakdown has been recorded in terms of all the components of this complex breeding system, but usually comprises a relaxation in the expression of self-incompatibility. This may significantly alter reproductive assurance regardless of the availability of pollinators and morph types, with positive consequences for at least the short-term future survival of rare species.

Potential factors that may contribute towards rarity in *Oxalis* were investigated by focusing on the reproductive biology and ecology of two widespread and eight rare/highly localized *Oxalis* species. The expression of tristylly, levels of natural seed production and vegetative reproduction were investigated to identify possible limitations to reproductive success. Several ecological attributes, including habitat type and specificity, population size and density, rainfall patterns, potential pollinators, potential threats, etc. were investigated for the studied populations and compared to the reproductive biological expression in each case. In addition, phylogeographic patterns were assessed by investigating the degree of genetic variation within and among populations of a well-supported monophyletic clade including two rare and highly localized species and one widespread species.

Comparisons of results of different rare species together with comparisons between rare and related common species are presented to reveal the most likely threats to specific populations. Tristylly expression was unstable and extremely variable among the populations studied. In addition to limited reproductive success, most rare species are restricted by their highly specific habitat requirements and are particularly vulnerable to variation in rainfall patterns. Low genetic variation within rare species may also prevent the colonisation of new habitats or the adaptation to a changing environment.

OPSOMMING

Vyf en twintig persent van alle suider Afrikaanse *Oxalis* spesies is skaars of bedreig met 'n baie gelokaliseerde verspreiding wat hulle veral kwesbaar maak vir gevare geassosieer met klein, geïsoleerde populasies (inteling, lae genetiese variasie, versteurde biologiese interaksies) wat tot uitsterwing kan lei. Verder vertoon *Oxalis* tristylie, 'n beperkende kruisingsstelsel wat 'n sterk self-onverenigbaarheidskomponent insluit om uitteling binne populasies te bevorder. Omdat gelyke beskikbaarheid van drie verskillende morfologiese blomtipes en effektiewe bestuiwers vir saadproduksie nodig word, kan hierdie kruisingsstelsel 'n verdere bedreiging wees vir klein, geïsoleerde populasies indien dit ten volle uitgedruk word. Die afbreek van al die komponente van hierdie komplekse kruisingsstelsel is al gedokumenteer, maar behels meestal 'n verflouing in die uitdrukking van self-onverenigbaarheid. Dit kan die versekering van reproduksie aansienlik verbeter ongeag die beskikbaarheid van bestuiwers en blomvormologiese tipes, met positiewe nagevolge vir ten minste die korttermyn oorlewing van skaars spesies.

Potensiële faktore wat tot skaarsheid in *Oxalis* kan bydra is ondersoek deur te fokus op die voortplantingsbiologie en ekologie van twee wydverspreide en agt skaars/baie gelokaliseerde *Oxalis* spesies. Die uitdrukking van tristylie, vlakke van natuurlike saadproduksie en vegetatiewe voortplanting is ondersoek om moontlike beperkings tot voortplantingsukses te identifiseer. Verskeie ekologiese eienskappe wat habitat-tipe en -spesifiekheid, populasie-grootte en -digtheid, reënvalspatrone, potensiële bestuiwers, moontlike bedreigings ensovoorts insluit, is vir die bestudeerde populasies ondersoek en vergelyk met die voortplantingsbiologiese uitdrukking in elke geval. Verder is filogeografiese patrone bestudeer deur die graad van genetiese differensiasie binne en tussen populasies van 'n goed ondersteunde monofiletiese klade, wat twee skaars en gelokaliseerde spesies en een wydverspreide spesie insluit, vas te stel.

Die vergelyking van resultate van verskillende skaars spesies tesame met vergelykings tussen skaars en verwante vollop spesies word voorgedra om die mees waarskynlike bedreigings vir spesifieke populasies te identifiseer. Die uitdrukking van tristylie was onstabiel en baie variërend tussen die bestudeerde populasies. Buiten beperkte voortplantingsukses word die meeste skaars spesies beperk deur hul baie spesifieke habitat vereistes en is veral kwesbaar vir variërende reënvalspatrone. Lae genetiese variasie binne skaars spesies kan ook die kolonisering van nuwe habitate of die aanpassing by 'n veranderende omgewing bemoeilik.

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CHAPTER 1

INTRODUCTION

In South Africa 3 268 plant species have been recorded as 'threatened with extinction'. Given its area, southern Africa has the highest concentration of threatened plant taxa in the world. Most of the threatened taxa are found within the Cape Floristic Region, an area constantly subjected to fragmentation and disturbance (Hilton-Taylor 1996). More importantly, this region has been identified as one of the world's biodiversity hotspots based on its unique plant diversity (Meyers *et al.* 2000).

A species may be considered rare if it comprises one or more of the following characteristics: (1) if it occupies a narrow geographical distribution range, (2) if it has highly specific habitat requirements, or (3) if it is found only in small populations. The rarest species are those with a combination of a limited geographical range, high habitat specificity and small population size (Primack 1993; Pullin 2002). Endangered species are species that are facing a very high risk of extinction in the wild in the near future (Golding 2002).

The question of why some species are rare and some species are common has become an important area of research in conservation biology. The ecological and biological attributes, evolutionary history and environmental factors of a species affect its distribution and abundance and cause some species or populations to be more vulnerable to extinction than others (Burne *et al.* 2003).

Habitat fragmentation and disturbance

Since there is an intimate link between species and their habitats, the rapid rates of environmental change imposed by current human activity are threatening an increasing number of species which are unable to adapt to changing environments, pushing them towards extinction (Pullin 2002). Habitat fragmentation causes a reduction in population size and an increase in isolation, which may be linked to increased inbreeding, decreased individual fitness, loss of genetic variation, and consequently to increased risk of population extinction (Aizen *et al.* 2002; Pullin 2002). The effect of habitat fragmentation may not be uniform among species, but may depend on the life history of the individual species. It does not only affect rare species, but may also reduce survival probabilities

of more common species (Hooftman *et al.* 2003). Population declines into small and isolated units, the loss of populations associated with habitat transformation, and the prevailing land use between remaining populations, all potentially reduce inter-population gene flow, which results in a loss of the genetic variability of a species (Primack 1993; Young *et al.* 1996). Severe reductions in population size at the time of fragmentation can create genetic bottlenecks, where remaining individuals contain only a small sample of the original gene pool. Such populations have a high vulnerability to environmental changes and stochastic events (Primack 1993; Young *et al.* 1996). A special category of bottleneck, known as the 'founder effect', occurs when only a few individuals survive and manage to establish a new population (Primack 1993).

Species distribution

Species with a narrow distributional range or highly specific habitat requirements can be severely affected by habitat fragmentation, as the expansion of remaining populations is limited by a lack of suitable, high quality habitats. Even a single threatening event can affect all the individuals of the species. Such species are at a high risk of extinction, regardless of the quality of their seed production or any other biological attributes, especially if they are required to increase their population sizes in order to persist (Pantone *et al.* 1995; Murren 2002). A species that comprises of only a few populations is also at great risk of extinction, as the loss of any one population by habitat transformation or any chance factors may result in the loss of a high proportion of the remaining genetic variation of the species (Primack 1993).

Small population size

It is well-known that small populations are particularly vulnerable to extinction (Luijten *et al.* 2000; Burne *et al.* 2003). Small populations are more susceptible to environmental and demographic stochasticity, and therefore at a high risk of extinction (Luijten *et al.* 2000).

Moreover a sufficiently large population size is important to maintain genetic variation, which in turn is essential in that it allows populations to adapt to a changing environment. As population size declines, the population tends to lose genetic variation just by chance through a process known as genetic drift. This phenomenon is the result of a change in allele frequencies from one generation to the next, with rare alleles being at great risk of being lost in each generation. In a large

population the loss of alleles through genetic drift is balanced by the appearance of new alleles through mutation (Primack 1993; Hooftman *et al.* 2003, 2004).

Another consequence of small population size is susceptibility to inbreeding, which arises from increased self-pollination, or when remaining potential mates are closely related through recent common ancestry in small populations (Young *et al.* 1996). High levels of inbreeding increase the probability of harmful, recessive alleles to be expressed in the homozygous form. This reduced heterozygosity may have deleterious effects on the individuals, causing a reduced average fitness of the population (inbreeding depression) (Primack 1993; Pullin 2002; Hooftman *et al.* 2003).

Species with small population sizes are also more vulnerable to disrupted biological interactions. The number and diversity of pollinators are normally lower in small habitat islands, since small plant populations are less attractive to pollinators. This may cause pollen limitation and lower seed set in insect-pollinated species and reduce the potential for intra- and inter-population gene flow (Luijten *et al.* 2000; Hooftman *et al.* 2003).

Plant specialization

Plant populations that are dependent on mutualism for reproduction (e.g. pollination and seed dispersal) are more susceptible to fragmentation and other forms of habitat disturbance (Aizen *et al.* 2002). The survival of species that are highly specialized for pollination by specific pollinators is highly dependent on the survival of their pollinators. They face a great risk of extinction should their pollinator disappear. The Cape flora is replete with examples of specialized pollination systems (Linder 2003). For example, a large guild of plant species with red flowers is exclusively dependent on the mountain pride butterfly (*Meneris tulbaghia*) for pollination (Johnson and Bond 1994; Pauw and Johnson 1999). Extreme specialization is evident in a guild of 20 plant species dependent on a single long-tongued fly species (Manning and Goldblatt 1996; Johnson and Steiner 1997). Factors threatening the abundance of these keystone insect species also threaten a large number of plant species (Bond 1994).

Evolutionary history

Reduced population size and increased isolation lead to an erosion of genetic variation and increased inter-population genetic divergence. Such effects have serious implications for species persistence over time (Young *et al.* 1996).

The distribution and amounts of genetic diversity within and among populations of rare plants are likely to depend on whether a species has always been rare or whether it has recently become so as a result of human influences. Plant species that have a long history of being rare occur naturally in sparsely distributed and small populations, and they may even have adaptations that compensate for the disadvantage of rarity. In contrast, formerly widely distributed species that have experienced severe reductions in population sizes may be more susceptible to genetic stresses imposed by small population size (Falk and Holsinger 1991; Gustafsson and Sjögren-Gulve 2002). The most vulnerable species are those whose distribution patterns have been altered historically. These include rare endemics, formerly widely distributed and found in large populations, but due to severe reductions in their numbers now confined only to relicts of suitable habitat. Predictions of substantial climate change in the coming decades suggest that many plants will soon encounter similar restrictions (Falk and Holsinger 1991).

Breeding systems and rarity

Species with complex breeding systems can be more vulnerable to extinction as a consequence of certain requirements to retain outcrossing. Heterostyly is a breeding system that prevents inbreeding through the reciprocal arrangement of reproductive organs in different floral morphs, a physiological self-incompatibility system and through a suite of ancillary morphological features (Barrett 1990; Faivre and McDade 2001). Tristyly represents a complex form of heterostyly where three floral morphs (short, mid and long according to stigma height) are found within a population. Each plant possesses flowers with two anther levels that correspond to the stigma levels in the remaining two morphs (Figure 1). Seed production is normally restricted to pollination events between anthers and stigmas of equivalent height (legitimate pollinations), while pollinations between anthers and stigmas of different heights (illegitimate) usually fail to produce any seeds (Charlesworth 1979; Barrett 1988; Weller 1992). In addition to trimorphism in style and stamen length, there may be an array of ancillary characters, including trimorphisms of stigmatic papillae lengths, variation in pollen shape and size and the number of pollen grains produced per anther

(Ornduff 1964; Barrett 1990). The function of these morphological polymorphisms is to promote legitimate pollination, resulting in out-crossing that reduces the negative effects of close inbreeding in plant populations (Barrett and Glover 1985; Barrett 1992).

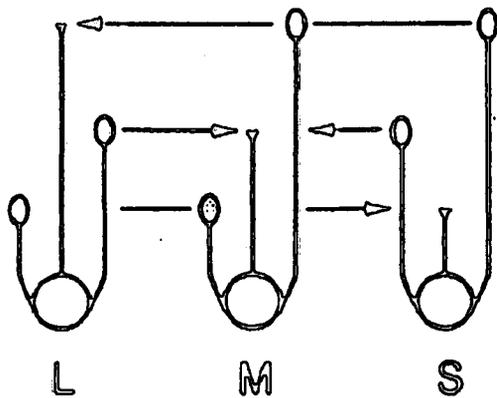


Fig. 1. A schematic diagram of the relative positions of styles and stamens in the three floral morphs of a tristylous species. Legitimate pollinations are indicated by arrows (Barrett 1989). L = long morph, M = medium morph and S = short morph.

These morphological trimorphisms favoring cross-pollination are accompanied by a physiological incompatibility system, which prevents fertilization following illegitimate pollination (Ornduff 1964; Glover and Barrett 1983). It is suggested that by promoting legitimate pollination, the morphological trimorphisms tend to prevent pollen wastage by ensuring that pollen is deposited onto the corresponding stigmas (Yeo 1975).

The tristylous breeding system is generally well-documented in only three families of flowering plants, namely the monocotyledonous Pontederiaceae, and the dicotyledonous Oxalidaceae and Lythraceae in which it has evolved independently (Ornduff 1974; Barrett 1979; Charlesworth 1979). More recently it has also been reported in the Amaryllidaceae, Connaraceae and Linaceae (Barrett *et al.* 1997; Graham and Barrett 2004). A system with two loci, S and M, each with two alleles and S epistatic to M, has been reported for the genetic control of tristily. With this genetic control, a 1:1:1 ratio of floral morphs will succeed in large tristylous populations at equilibrium when all morphs have equal fitness. When such a ratio occurs, the population is said to be at isoplethic equilibrium (Barrett 1992; Richards and Barrett 1992). Skewed morph ratios in small populations would reduce available compatible pollen for stigmas of the dominant morph type. For tristily to function effectively, populations should therefore contain all three floral morphs (Ornduff 1964), and an effective pollen vector is needed for the efficient transfer of pollen between different

morph types. This breeding system can therefore be restrictive to sexual reproduction in small, isolated populations where these requirements are often not met (Brys *et al.* 2004).

Numerous examples of modifications and break down of the tristylous breeding system have been reported. The most common pathway involves relaxation and eventual loss of the self-incompatibility system, followed by modifications in the relative heights of stigmas and anthers (Ornduff 1972; Barrett 1979; Weller 1992). Within small populations skewed morph frequency ratios and the more severe absence of one or more morph types can be attributed to founder effects or habitat fragmentation. As a result, such populations contain a limited number of genotypes that will not necessarily contain all four alleles required for the presence of the three morphs at equilibrium (Ornduff 1964; Morgan and Barrett 1988). Genetic drift within small populations may also result in a reduction of diversity of alleles (Luijten *et al.* 2000). The loss of stylar morphs from tristylous populations is often accompanied by evolutionary modifications to the remaining floral morphs, which may include changes to incompatibility and/or alterations in organ heights to increase the chances of their pollen reaching a receptive stigma (Graham and Barrett 2004). Over the short term, self-compatibility may thus be advantageous within very small populations where cross-pollination is insufficient as it enables seed production regardless of population structure and pollinator availability. Reproductive assurance may therefore be more important for some species than the genetic variability attained by cross-pollination (Barrett 1977, 1985). Studies by Pantone *et al.* (1995) indicates that by favoring homostyly, the weedy *Amsinckia menziesii* var. *intermedia* Fischer and Meyer overcame the intrinsic limitations of fecundity as presented by heterostyly in the rare species *A. grandiflora* Gray. However, the combination of small population size and a loss of self-incompatibility can be especially severe in reducing genetic variation (Hooftman *et al.* 2003, 2004).

The genus *Oxalis*

Oxalis L., *Biophytum* DC. and *Averrhoa* L. are the only three oxalidaceous genera that exhibit heterostyly, with tristylous restricted only to the genus *Oxalis* (Dreyer, 1996). *Oxalis*, consisting of almost 900 species, is the largest and most diverse genus in the Oxalidaceae. It has two centers of diversity, one in southern Africa and another in South-Central America (Salter 1944; Lourteig 1994, 1995, 2000).

There are *ca.* 210 species (*ca.* 270 taxa) of *Oxalis* in southern Africa (Dreyer and Makgakga 2003), with most species concentrated in the southern and southwestern parts (winter rainfall area) of the Western Cape Province, forming a distinct centre of diversity in this region (Salter 1944; Oberlander *et al.* 2002). This area is included in the very unique and widely discussed Cape Floristic Region (CFR) (Goldblatt 1978; Goldblatt and Manning 2000). *Oxalis* is the seventh largest genus within the Cape Flora (Goldblatt and Manning 2000), but an estimated 53 of the 210 (*ca.* 25%) southern African *Oxalis* species are rare/endangered and highly locally distributed (Hilton-Taylor 1996). While *Oxalis* species are found on a diversity of soil types, the genus is most diverse on shale-derived, clay soil (Linder 2003). Although *Oxalis* is among the most prolific of the southern African genera in terms of number of species and varieties, it has received limited systematic or ecological study in recent years. It is perhaps true that all the African species are morphologically tristylous as Salter (1944) suggested, but very little is known about the true state of tristyly expression among southern African species.

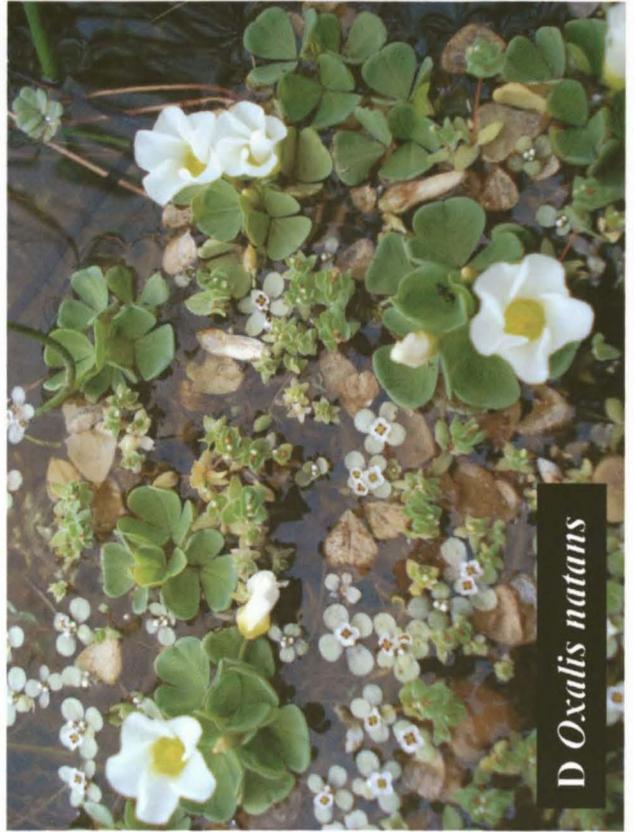
In addition to sexual reproduction, southern African *Oxalis* also reproduces vegetatively by producing bulbils from an underground rhizome (Ornduff 1974; Dreyer 1996). These bulbils are produced both within the old bulbs, and/or in the axils on the subterranean rhizome present in all indigenous southern African *Oxalis* species. Populations in very disturbed areas preferentially reproduce vegetatively by increasing the number of bulbs. This might be an attempt to counter the unsuitable environmental conditions for seedling establishment (Gebremariam 2004). The efficient ability to reproduce vegetatively may be beneficial in the short term in small isolated populations where seed production is restricted by tristyly. Over the long term, population persistence and expansion is normally still dependent on seedling production, due to evolutionary implications of asexual reproduction (loss of genetic diversity, accumulation of deleterious mutations, higher genetic loads and inbreeding depression) (Burne *et al.* 2003).

Objectives of this study

The main objective of this study was to investigate potential factors that may contribute towards rarity in selected rare/endangered southern African *Oxalis* species. This was achieved both through an ecological (including reproductive biological) and a molecular approach. Such a multi-faceted, comparative approach should aid our understanding of the processes that cause(d) rarity in *Oxalis* and thus aid future conservation attempts.



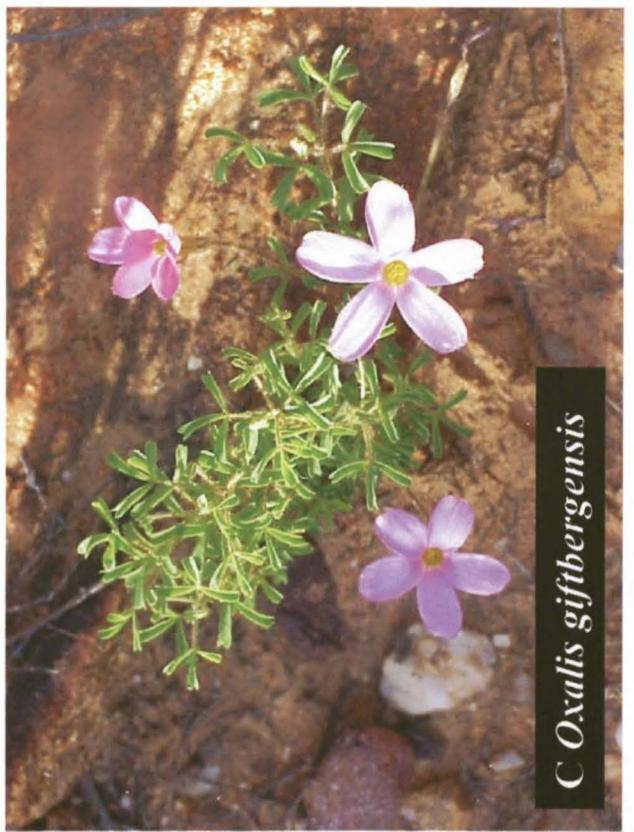
B Oxalis comptonii



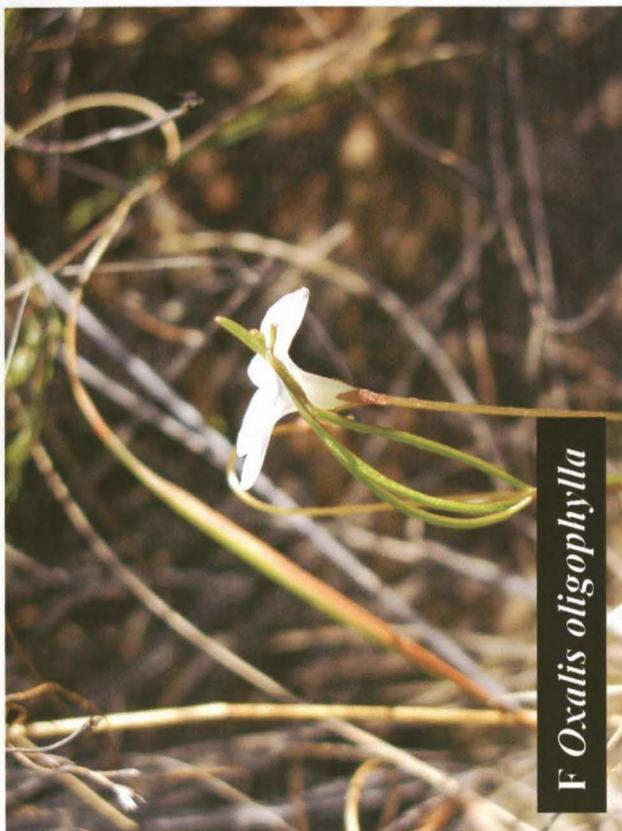
D Oxalis natans



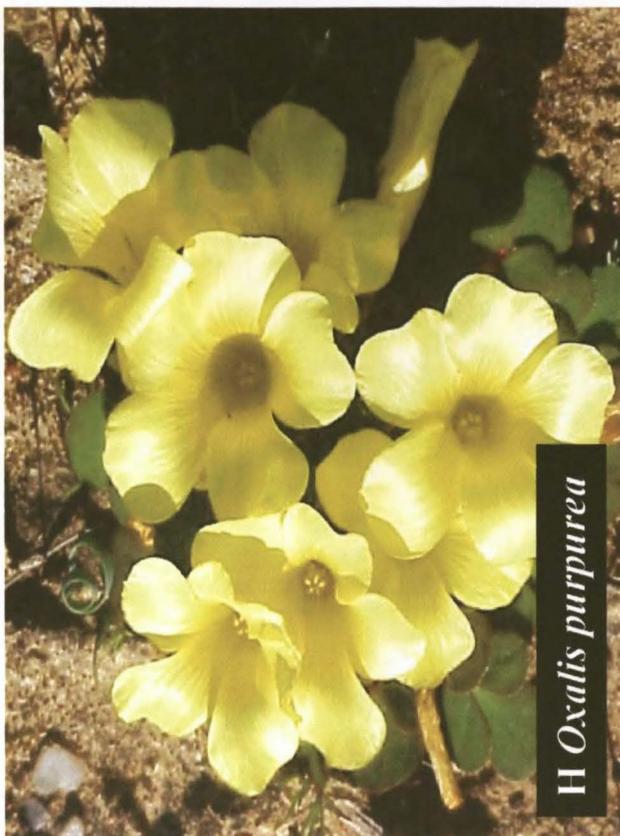
A Oxalis amblyosepala



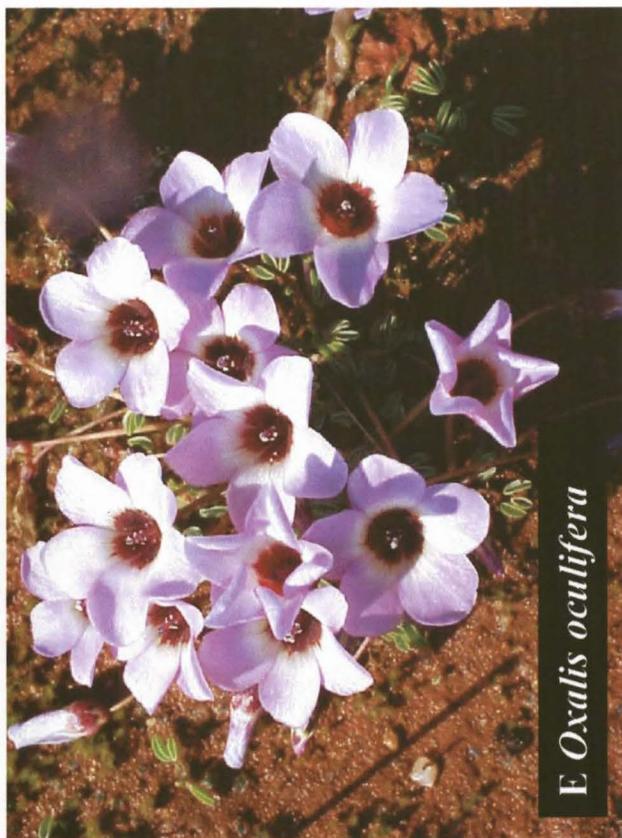
C Oxalis gifbergensis



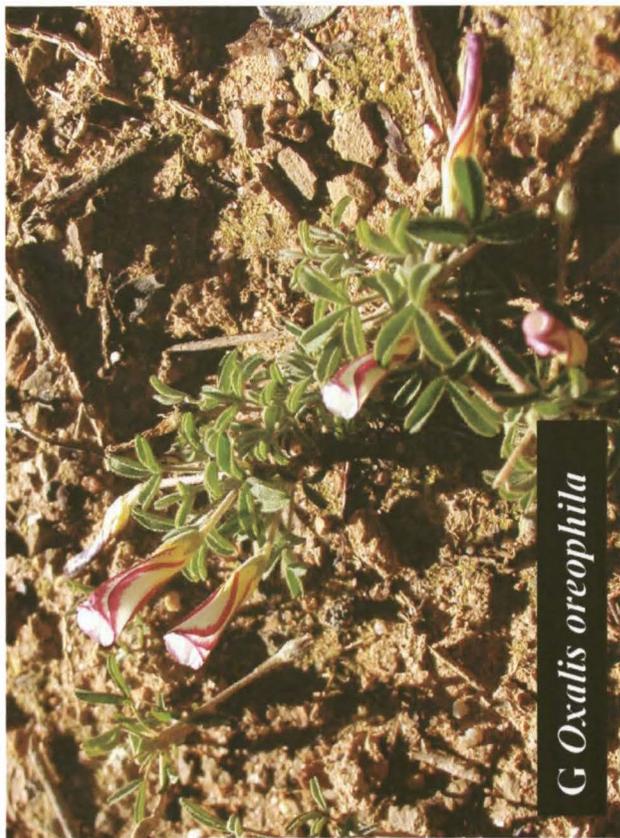
F *Oxalis oligophylla*



H *Oxalis purpurea*



E *Oxalis oculifera*



G *Oxalis oreophila*



Fig. 2. A) *O. amblyosepala*, B) *O. comptonii*, C) *O. giftbergensis*, D) *O. natans*, E) *O. oculifera*, F) *O. oligophylla*, G) *O. oreophila*, H) *O. purpurea*, I) *O. tomentosa* (Photo taken by Bill Dijk), J) natural habitat of *O. comptonii* and *O. oculifera*, K) natural habitat of *O. natans*. Photographs taken by J. Zietsman

The ecological component (Chapter 2) of this study focuses on the rare/endangered species *O. amblyosepala* Schltr., *O. comptonii* Salter, *O. giftbergensis* Salter, *O. hygrophila* Dreyer, *O. natans* L. f., *O. oculifera* E.G.H. Oliver, *O. oligophylla* Salter and *O. oreophila* Salter, and two widespread species (*O. purpurea* L. and *O. tomentosa* L.f.) that are included as controls (see Figure 2). The first objective was to assess several aspects of their reproductive biology (the expression of tristily, levels of seed production in the wild and vegetative reproduction) to determine to what extent reproductive success of the selected rare/endangered *Oxalis* species are limited by their reproductive biological attributes. The second objective was to determine the most likely ecological threats to the survival of the selected rare/endangered species.

As the third objective (molecular component) we aimed to determine the phylogeographic population structure of a well-supported monophyletic lineage that includes the widespread species *O. tomentosa* as sister taxon to the rare species-pair *O. hygrophila* and *O. oligophylla* (Chapter 3). This chapter has been submitted for publication and follows the format of Taxon.

An overview comparison of results of the different rare species together with comparisons between rare and related common species should help to identify the most likely reasons for species' small population sizes and/or restricted distribution. Combined conclusions are presented in Chapter 4.

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CHAPTER 2

The reproductive biology and ecology of selected rare and endangered
Oxalis L. (Oxalidaceae) species

Abstract

Twenty five percent of all southern African *Oxalis* species are rare/endorsed and highly localized making them especially vulnerable to extinction through inbreeding, low genetic variation, disrupted biological interactions, vulnerable to stochastic events, etc. – all consequences of small population sizes. Moreover *Oxalis* displays tristylly, which is a restrictive breeding system that includes a strong self-incompatibility component that promotes out-crossing within populations. As it requires equal availability of three floral morphs as well as effective pollinators for seed production, this breeding system can be a further threat to small populations when fully expressed. Potential factors that may contribute towards rarity in *Oxalis* were investigated by focusing on the expression of tristylly, levels of natural seed production, clonality and the ecology of two widespread and eight rare/highly localized *Oxalis* species. Field experiments revealed that the reproductive success of some *Oxalis* species is limited by the complex tristylous breeding, which results in extremely low levels of seed production in the wild. Some species overcome these limitations by a relaxed expression of tristylly that provides reproductive assurance regardless of population structure and pollinator availability. Others are rare and endorsed, but conditions still appear to be suitable to retain the characteristics of the tristylous breeding system. Most species are limited by their highly specific habitat requirements and are particularly vulnerable to variation in rainfall patterns.

Keywords: habitat specificity, *Oxalis*, rarity, small population sizes, tristylly expression

INTRODUCTION

The South African flora includes 3268 recorded threatened plants species, which, given the area of the country, gives South Africa the highest concentration of threatened plant taxa in the world. Most of these taxa are found in the Cape Floristic Region (CFR) (Hilton-Taylor, 1996). *Oxalis* is the seventh largest genus in the CFR (Goldblatt and Manning, 2000), but an estimated 53 of the 210 southern African *Oxalis* species are rare/endangered and highly locally distributed (Hilton-Taylor, 1996).

Rare species can be defined as species with a narrow geographical distribution range, highly specific habitat requirements and/or species restricted only to small populations (Primack, 1993; Pullin, 2002). This definition characterizes several of the southern Africa *Oxalis* species (Hilton-Taylor, 1996). Small populations are particularly vulnerable to extinction due to vulnerability to environmental stochasticity, low genetic variability caused by genetic drift and inbreeding depression which may reduce individual fitness (Primack, 1993; Pullin, 2002). Furthermore, small, isolated populations often suffer from disrupted biological interactions (Luijten et al., 2000; Hooftman et al., 2003). In addition to these problems imposed by small population sizes, complex breeding systems may also cause some species to be more vulnerable to extinction than other species with less complex or restrictive breeding systems.

All the southern African members of *Oxalis* displays tristylly (Salter, 1944), which is a form of heterostylly where three different morph types (Long, Mid and Short, according to stigma height) are present within a population. Reproductive organs are reciprocally arranged such that each morph type possesses flowers with two anther levels that correspond to the stigma levels in the remaining two morphs (Fig 1). This morphological trimorphism, together with a strong self-incompatibility component, enforces out-crossing in that seed production is limited only to legitimate crosses (between stigmas and anthers of equivalent heights). Enforced out-crossing should reduce the negative effects of close inbreeding in plant populations (Ornduff, 1964; Weller, 1992). A genetic control of a two loci system ensures that a 1:1:1 ratio of the floral morphs will succeed in large populations at equilibrium. When such a ratio occurs, the population is said to be at isoplethic equilibrium (Barrett, 1992; Richards and Barrett, 1992). For tristylly to function effectively, it requires equal availability of the three morph types as well as an effective pollen vector to transfer pollen between morph types. This breeding system could therefore be restrictive to sexual reproduction in small populations in which these requirements are often not met (Brys et al., 2004). Several studies have shown modifications and break down

in one or more of the components of the tristylous breeding system (Ornduff, 1972; Barrett, 1979; Charlesworth, 1979; Weller, 1976, 1992; Graham and Barrett, 2004). As a result, a relaxed self-incompatibility could provide reproductive assurance regardless of pollinator and mate availability and could therefore aid in the short term survival of rare populations. In addition to sexual reproduction, all southern African *Oxalis species* also reproduce vegetatively by producing bulbils from an underground rhizome (Ornduff, 1974; Dreyer, 1996) which may counter unsuitable conditions for seed production and/or seedling establishment.

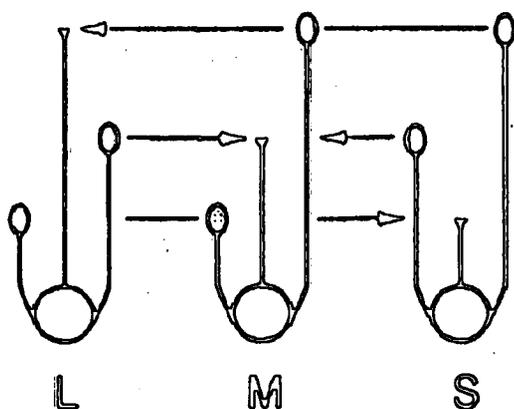


Fig. 1. A schematic diagram of the relative positions of stigmas and anthers in the three floral morphs of a tristylous species. Legitimate pollinations are indicated by arrows (after Barrett, 1989). L = long morph, M = medium morph and S = short morph.

The present study set out to assess the factors that control rarity in selected southern African *Oxalis* species, by considering I. reproductive biology and II. ecological parameters of each of these species.

I. Reproductive Biology

The first objective was to determine to what extent the reproductive success of selected rare/endangered *Oxalis* species are limited by their reproductive biology by assessing the expression of tristily, levels of natural seed production and vegetative reproduction (clonal growth). The breakdown of one or more components of the tristylous breeding system, which may restrict sexual recruitment in small populations, may significantly alter reproductive assurance and thus survival of a species. Three aspects of tristily, namely self-incompatibility, population structure (morph frequency ratios) and flower morph morphology were therefore assessed.

Levels of natural seed production (seed production in the wild) were explored to determine if sexual reproduction is restricted within a population. To test if seed production is specifically constrained by the tristylous breeding system, natural seed production was compared to seed production following controlled, legitimate cross-pollinations. It was hypothesized that within small, self-incompatible populations, natural seed production would be much lower than in controlled cross-pollinated flowers due to restrictions associated with tristylous. Low seed production could, however, also be a result of lower fecundity as a consequence of inbreeding depression in small populations (Hooftman et al., 2003). In this case seed production in controlled crosses would also be lower than expected. Where morph ratios are highly skewed, natural seed production was expected to be higher in the deficient morph type, because it has proportionally more legitimate mates in the population than the more common morphs (Årger, 1996).

The extent of clonality was also assessed to determine if the population is solely dependant on seed production for survival, or whether the sexual reproduction bottleneck could be countered by vegetative propagation. The extent of clonality could be either beneficial or harmful to the short-term survival of a rare species, depending on the level of self-incompatibility expression in the particular species. If a rare species is fully self-incompatible, extensive clonal propagation would further threaten population (and possibly species) survival, as it would result in skewed morph frequency ratios, and thus reduce the possibility for natural seed formation (Ornduff, 1964; Barrett, 1977). If, however, a rare species is self-compatible, skewing of morph frequency ratios does not negatively affect seed production. In fact, extensive clonal growth could increase the number of potentially seed-producing individuals, and thus positively contribute to increased population sizes. Low levels of clonality would therefore favor self-incompatible rare species, while high levels of clonality would favor self-compatible species over the short term. Over the longer term very high levels of clonality would obviously lead to reduced genetic diversity.

II. Ecology

A second objective was to determine the most likely ecological threats to the survival of the selected rare/endangered species *O. amblyosepala*, *O. comptonii*, *O. giftbergensis*, *O. hygrophila*, *O. natans*, *O. oculifera*, *O. oligophylla* and *O. oreophila*. Several abiotic and biotic attributes were explored and compared to the reproductive biology of each species. For comparative purposes we also included two widespread species (*O. purpurea* and *O. tomentosa*) that are abundant in the Western Cape in all aspects of this study. We identified the most likely

reasons for the small population sizes of rare and/or restricted species by comparing these ecological traits/threats among the different rare species and also between rare and related common species.

MATERIALS AND METHODS

Study species

The selected *Oxalis* species (Table 1) share similar traits in terms of their natural history. They are all reportedly tristylous, insect pollinated, have the ability to reproduce vegetatively through bulbil formation and share a similar geophyte growth-form (Salter, 1944). Apart from these similarities, each rare species exhibits taxonomic and ecological differences that distinguish them both from the more common or other rare species. Rarity may thus be a unique response of each of these species to their unique environmental conditions as discussed by Murray et al. (2002).

Table 1. Species selected for comparisons of reproductive biological and ecological attributes.

Species	Flowering period	Study site	Distribution range
<i>O. amblyosepala</i>	May–Jul	Giftberg	Restricted
<i>O. comptonii</i>	May–Jun	Giftberg	Restricted
<i>O. giftbergensis</i>	May–Jun	Giftberg	Restricted
<i>O. hygrophila</i>	Nov	Pakhuis Pass	Restricted
<i>O. natans</i>	Sept–Nov	1. Stellenbosch 2. Elandsberg	Restricted
<i>O. oculifera</i>	May–Jun	Giftberg	Restricted
<i>O. oligophylla</i>	May–Jun	Giftberg	Restricted
<i>O. oreophila</i>	May	Pakhuis Pass	Restricted
<i>O. purpurea</i>	May–Sept	1. Stellenbosch 2. Piekenierskloof Pass 3. Uniondale	Widespread
<i>O. tomentosa</i>	Apr–Jun	1. Darling 2. Saron 3. Elandsberg	Widespread

Five of the selected rare/endangered species, *O. amblyosepala*, *O. comptonii*, *O. giftbergensis*, *O. oculifera* and *O. oligophylla*, are endemic to the Giftberg (Vanrhynsdorp), while *O. hygrophila* and *O. oreophila* are restricted to the Pakhuis Pass (Clanwilliam). *Oxalis natans* is an aquatic species restricted to remnants of vernal pools (seasonally aquatic habitats) in the

Western Cape. For this species we studied one highly disturbed population (Stellenbosch) and one natural population (Elandsberg) (Table 1). Three populations were included for each of the widespread species. *O. purpurea* is a weedy species that is very abundant in the Western Cape. It displays three flower colour forms (pink, yellow and white), with the pink form being most common in the Cape Peninsula, while the distribution of the yellow and white forms extend much further north towards Clanwilliam and Nieuwoudtville. *O. purpurea* was studied in Stellenbosch (*O. purpurea* 1), Piekenierskloof Pass (*O. purpurea* 2) and near Uniondale in the Southern Cape (*O. purpurea* 3). *Oxalis tomentosa* is also fairly common in the Western Cape and was studied in Darling (*O. tomentosa* 1), Saron (*O. tomentosa* 2) and Elandsberg (*O. tomentosa* 3). See Fig. 2 for the geographic localities of the study sites for the species included in this study.

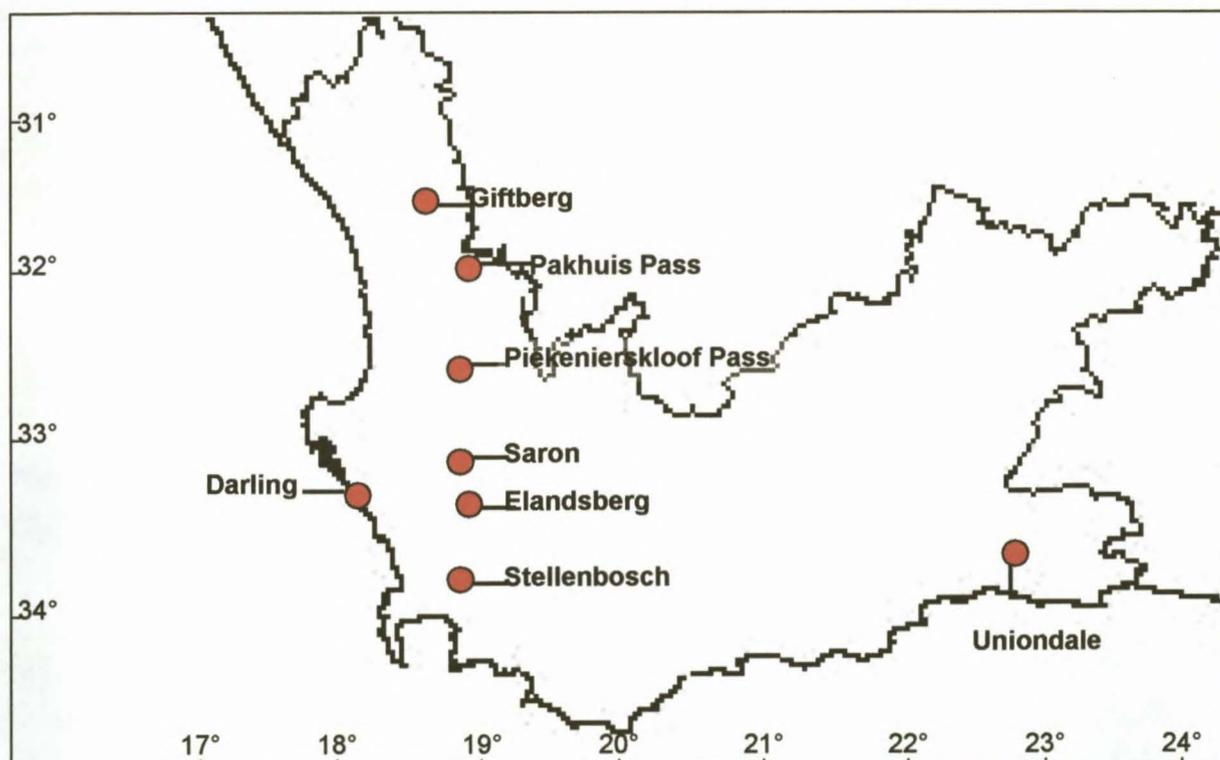


Fig. 2. Geographic locality of the sites in the Western Cape where the different rare and/or widespread *Oxalis* species were studied.

I. REPRODUCTIVE BIOLOGY

The reproductive biology of all the selected populations of all species was studied, except for the *O. hygrophila* population for which no flowering plants were found during the two year study period. The limited numbers of flowering plants and the short flowering period of certain species resulted in some crossing-experiments not being performed in all populations. These

included assessments of self-incompatibility expression (*O. oligophylla* and *O. tomentosa*) and natural seed production (*O. oligophylla*).

Tristyly Expression

Field-based artificial pollinations were performed to assess the expression of self-incompatibility in the selected species. Seed production following controlled self-pollinations was compared to seed production following legitimate cross-pollinations (control), where seed production is expected to be at a maximum. Ten replicates of each cross-combination (Table 2) were performed randomly in the field.

Flowers were emasculated on the first day of anthesis, where after pollen from the desired anther whorl was rubbed onto the stigma using fine forceps. Artificially pollinated flowers were bagged with fine netting (emptied teabags) to avoid uncontrolled pollination. The tiny seeds mature 14 to 28 days after pollination (J. Zietsman, personal observation), and are explosively shed from the capsule at maturity through rupture of the outer testa layer (Salter, 1944). Mature fruits were collected and the percentages of flowers producing seed were determined for each of the different floral morphs per pollination treatment. The index of self-incompatibility (ISI) was expressed as the ratio of the number of fruits formed following manual self-pollination relative to the number formed following manual cross-pollination, following Zapata and Arroyo (1978).

Table 2. The pollination types performed represented as selected stylar morph (♀) x specific anther level/style morph (♂). L, M & S represent stylar morphs and s, m & l represent anther levels.

Selfing		Legitimate crosses	
L x s/L	L x m/L	L x l/S	L x l/M
M x s/M	M x l/M	M x m/S	M x m/L
S x m/S	S x l/S	S x s/M	S x s/L

Data from experimental pollinations were analyzed by non-parametric statistics (Statistica software package) as many flowers did not set fruit, causing this variable to be non-normally distributed. Mann-Whitney *U*-tests were performed to compare selfing to legitimate cross-pollinations as well as to compare natural seed production (discussed below) to legitimate cross-pollinations. Kruskal-Wallis ANOVA *H* was used to test for differences between the three floral morphs for all pollination types.

Population structures were examined by determining the flower morph frequency ratio in each population. The floral morph types were recorded for at least 100 flowers (where possible) in each population during the peak flowering period of each species. Plants were sampled at one to two-meter intervals to avoid the sampling of clones. Morph frequency ratios were analyzed by *G* statistics (Sokal and Rohlf, 1995) for goodness-of-fit to an expected isoplethic (1:1:1) equilibrium. The reproductive organs of 20 randomly chosen flowers of each morph were measured to assess morph morphology. Stigma and anther heights were measured from the base of the ovary using calipers. Organ heights of individuals were plotted onto graphs to compare morphological alterations of tristily in different populations and species.

Natural Seed Production

Twenty-five randomly chosen flowers of each morph type were bagged in the wild after allowing sufficient time for natural pollination to have occurred (when petals start to dehisce). Seed production in these untreated flowers was compared to seed production following artificial legitimate cross-pollination where seed production is expected to be at a maximum. Natural seed production was also compared between different morphs of the same species to test if the more common morphs in non-isophletic populations are more likely to suffer from pollen limitation as discussed by Årgen (1996).

Vegetative Reproduction

The extent of clonality was studied by examining and quantifying bulbil formation on living material and available herbarium specimens obtained from the *Oxalis* collection in the Stellenbosch University Herbarium (STEU). In addition, the turnover of morph types was analyzed along transects, where 50 nearest-neighbor individuals per population were sampled. It was assumed that a high morph turnover would indicate low levels of clonality, as vegetative reproduction produces plants of the same morph type growing in close proximity to one another. The frequencies of two neighbors being of the same morph type vs. two neighbors being of different morph types were analyzed. *G* statistics (Sokal and Rohlf, 1995) was used to determine to what extent the ratio of the frequencies of two neighbors being of the same morph type vs. two neighbors being of different morph types deviates from 1:1. A ratio of 1:1 should indicate moderate levels of clonality, while a significant deviation towards the frequency of two neighbors being of the same morph type should indicate high levels of clonality. A significant deviation toward the frequency of two neighbors being of different morph types should indicate extremely low levels of clonality.

II. ECOLOGY

The geographical distribution of each species was determined using locality data obtained from the Compton (NBG) and Bolus (BOL) Herbaria and the Pretoria Computerized Information System (PRECIS) database. Population surveys were undertaken during which each species were evaluated for preferred habitat type and habitat specificity. The following habitat attributes were recorded: (1) Vegetation type; (2) Substrate; (3) Moisture regime; (4) Soil type; (5) Lithology; (6) Exposure; (7) Aspect; (8) Slope. Potential threats to available habitats were also evaluated. The ranges and available habitat of each population were recorded, as well as the extent of fragmentation and/or disturbance facing the populations. Population size and density were determined for all rare/endangered species and for selected populations of the two common congeners. Population sizes were estimated by multiplying plant density with population area. In addition, current and longer-term rainfall data were obtained from weather stations nearest to the selected study sites and compared to the current biological state of the populations. Pollinators were also monitored in terms of abundance and specificity to the selected species.

RESULTS

I. REPRODUCTIVE BIOLOGY

Expression of self-incompatibility and natural seed production

Results obtained from controlled pollination treatments of the three floral morphs of the selected populations are presented in Tables 3 and 4.

When tristylly is fully expressed self-pollinations should yield no seed, but this was only evident in *O. amblyosepala* and *O. natans*. In fact, controlled self-pollination experiments (Table 3) indicate a partial slippage in self-incompatibility for many populations, including those of *O. comptonii* and *O. oculifera* and especially the Uniondale population of the widespread *O. purpurea* (*O. purpurea* 3). In the latter population only self-pollinations of the L morph were possible, as the population consists only of this one morph type (Table 6).

For legitimate pollinations (pollinations between stigmas and anthers of the same height), seed production is expected to be at a maximum. For rare species, legitimate seed production was rather low compared to that of the widespread *O. purpurea*, but still markedly higher than seed production following self-pollination (Table 4).

Table 3. Seed production following controlled self-pollinations in selected *Oxalis* populations. Pollination treatments are presented as selected stylar morph (♀) x specific anther level/stylar morph (♂).

Species (population)	% Capsules						Total % (L,M,S)	Average number of seed/capsule
	L		M		S			
	L x sL	L x mL	M x sM	M x lM	S x mS	S x lS		
<i>O. amblyosepala</i>	0	0	0	0	0	0	0	0
<i>O. comptonii</i>	40	10	10	10	0	0	11.7	5.6
<i>O. giftbergensis</i>	0	0	0	10	0	0	1.7	1
<i>O. natans</i> (1)	0	0	0	0	0	0	0	0
<i>O. natans</i> (2)	0	0	0	0	0	0	0	0
<i>O. oculifera</i>	10	40	0	0	20	0	11.7	2.1
<i>O. oreophila</i>	0	10	0	0	10	0	3.3	2.5
<i>O. purpurea</i> (1)	0	10	0	0	30	0	6.7	2.1
<i>O. purpurea</i> (2)	0	20	10	0	10	0	6.7	2.3
<i>O. purpurea</i> (3)	80	90	*	*	*	*	85	22.5

* pollination treatments were not possible due to lack of morph types

Table 4. Seed production following controlled legitimate-pollinations in selected *Oxalis* populations. Pollination treatments are presented as selected stylar morph (♀) x specific anther level/stylar morph (♂).

Species (population)	% Capsules						Total % (L,M,S)	Average number of seed/capsule
	L		M		S			
	L x lM	L x lS	M x mL	M x mS	S x sL	S x sM		
<i>O. amblyosepala</i>	60	50	50	60	50	80	58.3	5.3
<i>O. comptonii</i>	60	30	90	50	20	70	53.3	7
<i>O. giftbergensis</i>	60	70	60	30	60	40	53.3	2.1
<i>O. natans</i> (1)	0	20	0	0	0	10	5	1
<i>O. natans</i> (2)	70	60	60	70	60	50	61.7	1.5
<i>O. oculifera</i>	90	80	80	60	40	60	68.3	3
<i>O. oreophila</i>	80	70	60	70	70	80	71.7	3.6
<i>O. purpurea</i> (1)	100	100	100	90	90	100	96.7	28.4
<i>O. purpurea</i> (2)	80	90	100	90	90	90	90	30.3
<i>O. purpurea</i> (3)	*	*	*	*	*	*	*	*

* legitimate pollinations not possible within monomorphic population

Combined results of the three morph types revealed that the total seed production following selfing (Table 3) was significantly lower than seed production following legitimate cross-pollinations (Table 4) as is expected in tristylous populations. *O. natans* 1 (Stellenbosch) was the only exception (Mann-Whitney *U* test, adjusted *Z* value 1.74679, *p* = 0.08067), in that both legitimate pollinations and selfing resulted in a very low percentage seed production (Table 3

and 4). When seed production following selfing is, however, compared between the different morphs of individual species, unique patterns were obtained for each species. *O. comptonii* and *O. oculifera*, for example, appear to be highly self-compatible for the L morph, and the percentage seed production differed significantly between the different morph types (Kruskal-Wallis ANOVA, $H = 6.043127$, $p < 0.05$; $H = 6.043127$, $p < 0.05$, respectively). Although the total percentage seed production (all three morph types combined) was significantly lower than percentage seed production following legitimate cross pollinations, the difference between these two pollination treatments was not significant when only the L morph for *O. comptonii* was considered (Mann-Whitney *U* test, adjusted *Z* value -0.978076 , $p = 0.328037$).

The importance of self-compatibility of the L morph in this population is further supported by the index of self-incompatibility (ISI) of 0.26 in *O. comptonii*, which is larger than the arbitrary proposed number of 0.20 required to characterize the population as partially self-compatible (Table 5). No statistical comparisons could be made for the highly self-compatible monomorphic population 3 of *O. purpurea*, as legitimate cross-pollinations could not be performed due to lack of available morph types. The ISI (% fruit selfing / % fruit legitimate cross) for this population was estimated by assuming the highest possible percentage fruit following legitimate cross-pollinations if more than one morph had been present. This resulted in a lowest possible ISI = 0.85, as legitimate fruit production any lower than 100 % would result in a higher value of ISI. The ISI for this population would therefore be > 0.85 , which falls within the arbitrary proposed range of 0.8–1 required to characterize a population as completely self-compatible.

There was extreme variation in the levels of natural seed production between the different populations studied (Table 5). Natural seed production was especially low in the completely self-incompatible *O. amblyosepala* and *O. natans* (1) and in *O. tomentosa* (1). Regardless of the low observed levels of natural seed production in many populations, only *O. amblyosepala*, *O. purpurea* (1) and *O. purpurea* (2) revealed significantly lower levels of natural seed production compared to seed production following legitimate cross-pollinations (Mann-Whitney *U* test, *O. amblyosepala*, adjusted *Z* value 2.955254, $p < 0.05$; *O. purpurea* 1, adjusted *Z* value 2.889628, $p < 0.05$; *O. purpurea* 2, adjusted *Z* value 2.106297, $p < 0.05$). For *O. natans* (1) both natural seed production and seed production following legitimate pollinations were extremely low. This comparison could not be performed for *O. tomentosa*, due to lack of sufficient material for controlled self- and legitimate cross-pollinations. There were no significant differences in seed production of different morph types for either legitimate cross-pollinations or natural seed

productions in the wild for any of the populations studied. Natural seed production was therefore not higher in the deficient morph type as observed by Årgen (1996) for the self-incompatible *Lythrum salicaria*.

Table 5. Seed production in natural pollinated flowers of selected *Oxalis* populations.

Species	Fruit set (%)			Total %	Average number of seed/capsule	ISI
	L	M	S			
<i>O. amblyosepala</i>	31.3	27.3	40	32.9	4.5	0.00
<i>O. comptonii</i>	76	60	60	65.3	8	0.22
<i>O. giftbergensis</i>	64	72	60	65.3	2.5	0.03
<i>O. natans</i> (1)	4	12	0	5.3	1	0.00
<i>O. natans</i> (2)	45	50	55	50	1.5	0.00
<i>O. oculifera</i>	56	52	68	58.7	2.6	0.17
<i>O. oreophila</i>	84	72	80	78.7	2.9	0.05
<i>O. purpurea</i> (1)	72	84	84	80	23	0.07
<i>O. purpurea</i> (2)	76	68	84	76	24.3	0.07
<i>O. purpurea</i> (3)	70	*	*	70	25.7	>0.85
<i>O. tomentosa</i> (1)	0	0	0	0	0	*
<i>O. tomentosa</i> (2)	47	30	37	38	4	*
<i>O. tomentosa</i> (3)	50	50	22.7	40.9	5	*

* ISI not calculated as a result of insufficient material

The number of seeds per capsule varied between populations and depends on the capsule morphology that varies considerably between species. Species with endospermous seeds (e.g. *O. purpurea*) usually produce many seeds per capsule, while species with exendospermous seeds (e.g. all of the rare species that were studied) can only produce fewer seeds per capsule (often restricted to only 5 possible seeds per capsule) (Salter, 1944).

Morph frequency ratios

Among all the populations of the rare/endangered species, only *O. comptonii* and *O. oculifera* display a morph frequency ratio that deviates significantly from the expected 1:1:1 (isoplethic equilibrium) (Table 6). In addition, two of the three populations of *O. purpurea* and one of the populations of *O. tomentosa* studied are non-isoplethic (morph ratio deviates from 1:1:1). In general the L morph is under-represented in all of the populations, except in *O. purpurea* (2) and (3) where it predominates.

Table 6. Floral morph frequencies for selected *Oxalis* populations compared by a G test for goodness-of-fit to an isoplethic (1:1:1) equilibrium.

Species	n	Floral morph			G	Significance*
		L	M	S		
<i>O. amblyosepala</i>	105	33	37	35	0.229	NS
<i>O. comptonii</i>	51	14	11	26	7.081	P < 0.05
<i>O. giftbergensis</i>	104	33	40	31	1.265	NS
<i>O. natans</i> 1. (Stellenbosch)	100	30	31	39	1.425	NS
<i>O. natans</i> 2. (Elandsberg)	86	24	24	38	4.363	NS
<i>O. oculifera</i>	109	28	51	30	8.505	P < 0.05
<i>O. oligophylla</i>	56	12	26	18	5.317	NS
<i>O. oreophila</i>	94	28	27	39	2.736	NS
<i>O. purpurea</i> 1. (Stellenbosch)	150	42	54	54	1.978	NS
<i>O. purpurea</i> 2. (Piekenierskloof)	143	67	42	34	12.015	P < 0.05
<i>O. purpurea</i> 3. (Southern Cape)	103	103	0	0	226.309	P < 0.06
<i>O. tomentosa</i> 1. (Darling)	55	14	39	2	42.465	P < 0.05
<i>O. tomentosa</i> 2. (Saron)	96	25	43	28	5.589	NS
<i>O. tomentosa</i> 3. (Elandsberg)	120	45	38	37	0.933	NS

* Significance determined from Chi-square distribution

Morph morphology

When organ heights were plotted and individuals arranged in decreasing stigma height for each morph for all populations, considerable variation among individuals of the same morph was observed (Fig. 3 and 4). Gaps in the figures (*O. comptonii* and *O. oligophylla* – Fig. 3; *O. tomentosa* 1 and *O. purpurea* 3 – Fig. 4) indicate where less than 20 flowers were recorded for a particular morph type. Three trends are noticeable among populations of the rare species (Fig. 3). In the morphologically truly tristylous populations (*O. amblyosepala*, *O. giftbergensis* and *O. oreophila*) there is a clear discontinuation of stigma heights between the long and the mid, and the mid and short stigma heights and there are three clearly separated organ levels in each morph. A second trend shows a continuum of stigma heights between the different morphs (*O. natans* 1, *O. comptonii* and *O. oculifera*), but three separated organ heights are still present within each morph type. Thirdly, there are populations in which the styles are not of three distinct length classes, and this is coupled with an overlap in organ heights in the different morph types (*O. natans* 2 and *O. oligophylla*). *O. oculifera* and *O. natans* have very small flowers and the separation between organ heights appears to be much less than in the other species studied.

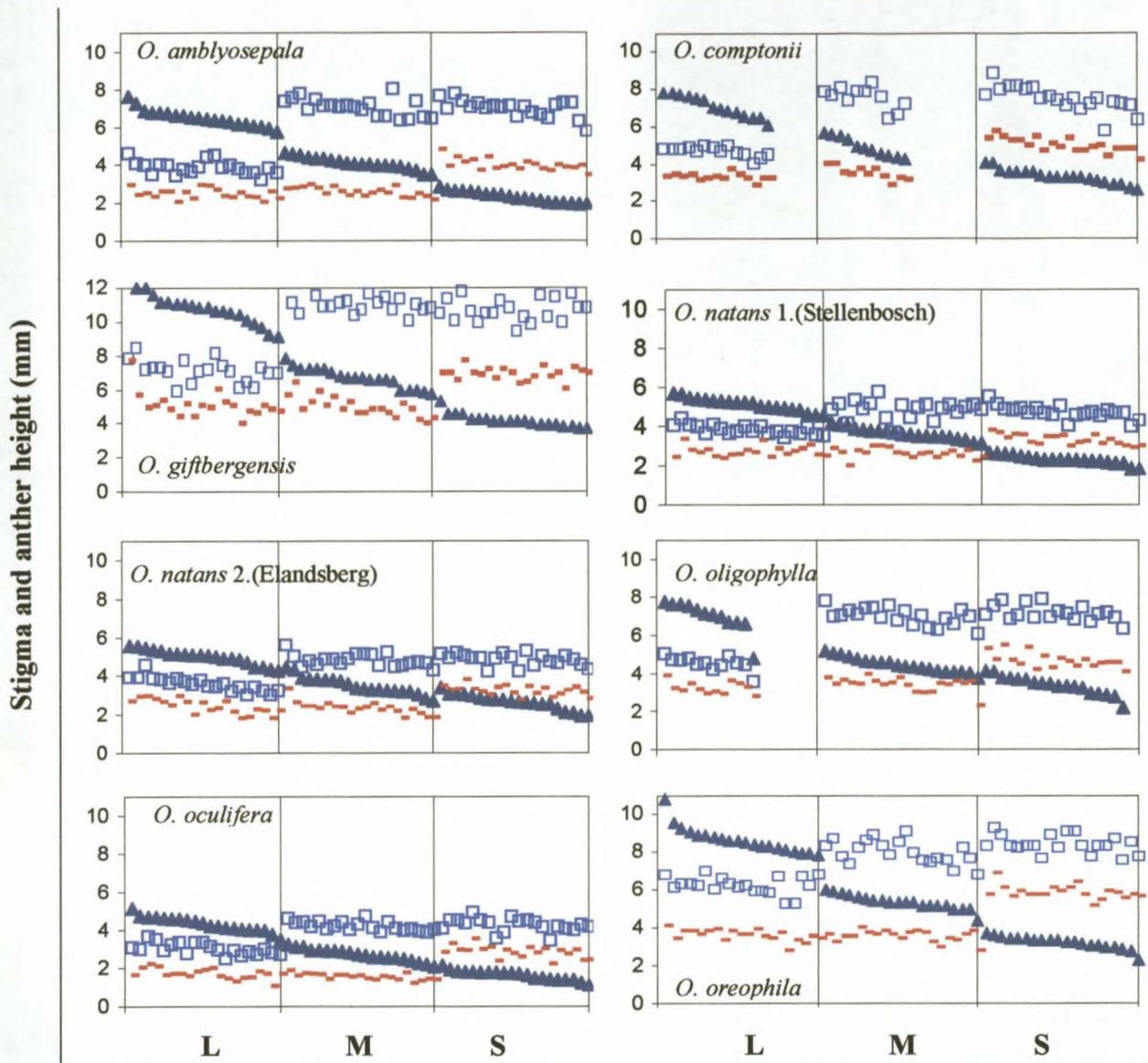


Fig. 3. Variation in the heights of stigmas and anthers in populations of rare/endangered *Oxalis* species. Individuals in each population are ordered by decreasing style length. (Style \blacktriangle , upper anthers \square , lower anthers $-$; L = long morph, M = mid morph, S = short morph)

The three populations of *O. tomentosa* appear to be morphologically tristylous (Fig. 4). Populations of *O. purpurea* vary in their morphological expression of tristily. *O. purpurea* (1) appears to be morphologically truly tristylous, while there is a continuum in stigma heights between the mid and short flowers of *O. purpurea* (2). In the latter population morphological alteration of some individuals of the short morph flowers were also observed. *O. purpurea* (3) is very interesting in that this population completely lacks two of the floral morphs and displays considerable morphological aberration within the remaining L morph. The height of the mid-level anthers have increased to approach the same height as the L stigma in some flowers, while the long styles have shortened to approach the same level as the mid-level anthers in other

flowers. These morphologically aberrant L morph flowers result in mid-level anthers and long-level stigmas being borne at the same level, which brings them into close proximity to each other.

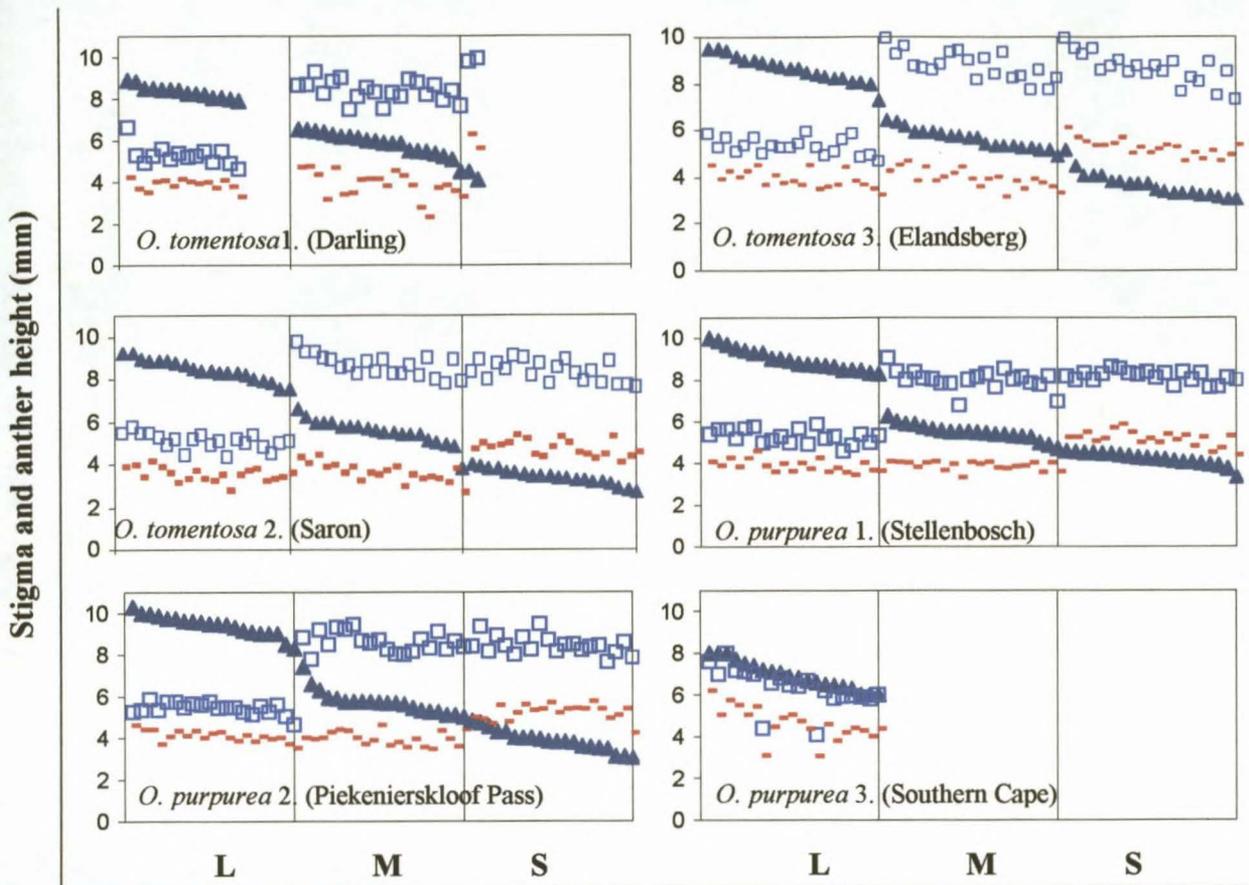


Fig. 4. Variation in the heights of stigmas and anthers in selected populations throughout the distribution range of the widespread/common *Oxalis* species. Individuals in each population are ordered by decreasing style length. (Style \blacktriangle , upper anthers \square , lower anthers $- -$; L = long morph, M = mid morph, S = short morph)

Vegetative reproduction

Field surveys indicate that vegetative reproduction is more common in the widespread species compared to the rare species (Table 7). Within populations of the widespread species, profuse vegetative reproduction via bulbil formation results in clumps or clusters of plants of the same morph type, while plants of the rare species grow in a more solitary fashion.

When frequencies of plants of similar morph type vs. plants of dissimilar morph types occurring next to each other in transects of 50 individuals were compared to 1:1, *G* statistics revealed no significant deviation for most of the rare populations. This suggests moderate levels of clonality

in the rare species. A strong deviation towards neighboring plants being of dissimilar morph types indicates low levels of clonal growth in *O. giftbergensis*. In contrast, a strong tendency towards neighboring plants being of the same morph type was evident for all of the widespread populations (except *O. purpurea* 2) studied, and in one population of the rare species *O. natans* (1). This suggests high levels of clonality within populations of the widespread species, and within the very disturbed population of *O. natans*.

Table 7. Frequencies of plants of similar morph type vs. plants of dissimilar morph types occurring next to each other along transects intersecting 50 individuals of each population. Frequencies are compared by a *G* test to 1:1 ratio (moderate levels of clonality).

Species	Frequency		G	Significance*	clonality
	similar morphs next to each other	dissimilar morphs next to each other			
<i>O. amblyosepala</i>	0.72	1.28	3.973	NS	moderate
<i>O. comptonii</i>	0.68	1.32	5.211	NS	moderate
<i>O. giftbergensis</i>	0.52	1.48	12.009	P < 0.05	low
<i>O. natans</i> (1)	1.52	0.48	14.207	P < 0.05	high
<i>O. natans</i> (2)	1.28	0.72	3.973	NS	moderate
<i>O. oculifera</i>	0.88	1.12	0.722	NS	moderate
<i>O. oligophylla</i>	0.68	1.32	5.211	NS	moderate
<i>O. oreophila</i>	1.00	1.00	0.000	NS	moderate
<i>O. purpurea</i> (1)	1.40	0.60	8.228	P < 0.05	high
<i>O. purpurea</i> (2)	0.76	1.24	2.908	NS	moderate
<i>O. purpurea</i> (3)	2.00	0.00	69.312	P < 0.05	high
<i>O. tomentosa</i> (1)	1.64	0.36	22.175	P < 0.05	high
<i>O. tomentosa</i> (2)	1.36	0.64	6.628	P < 0.05	high
<i>O. tomentosa</i> (3)	1.40	0.60	8.228	P < 0.05	high

II. ECOLOGY

Details of the geographical localities, habitat attributes and distributions of each population are summarized in Appendix 1. *O. amblyosepala*, *O. oculifera* and *O. comptonii* are Giftberg endemics found in very fragile and specific habitats. *O. amblyosepala* is restricted to Restionaceae-dominated flat plains, where it occurs only in seasonally waterlogged pockets of soil on and between flat sandstone rocks. It grows in small, locally dense patches with mosses and lichens. The flowering period of this species stretches from May to July. *O. oculifera* and *O. comptonii* co-occur in a very distinct habitat in the Giftberg, which is rather similar to the habitat of *O. amblyosepala*. They occur in small, seasonally waterlogged patches comprised of a lens of fine clay and moss on a solid layer of sandstone bedrock. These specific microhabitats trail along the trajectories of small streams that develop during the rainy winter months. Both species

have a very high local density (especially *O. oculifera*), a flowering period from May to July and grow in patches with mosses, ferns, lichens and *Drosera* species.

For *O. amblyosepala*, *O. comptonii* and *O. oculifera* the number and sizes of population patches appears to be highly dependent on rainfall and varied considerably between 2005 and 2006. During the wetter 2006, many new sub-populations were observed in similar available habitat patches that were not observed during May–July 2005. The total annual rainfall for 2006 (158 mm) was higher than for 2005 (104 mm). In addition, 2006 was characterized by extensive winter rainfall early in the season (85.5 mm during May), whilst winter rainfall was more evenly spread throughout the season (April–August) in 2005.

O. giftbergensis only occurs in refugia of arid fynbos on the Giftberg. It is locally abundant in large populations, but is restricted only to the Giftberg mountain slopes, which can be viewed as an island surrounded by succulent karoo shrublands. *O. oligophylla* is another Giftberg endemic that grows in Mountain Fynbos. It occurs in rock crevices high on the Giftberg escarpment, where it is present in extremely low densities. Both *O. giftbergensis* and *O. oligophylla* flower from May to June.

O. hygrophila has been recorded from a moist, natural water seepage band in the Pakhuis Pass. When visited in 2001, the site had been recently burnt and as in 1944 (when this species was first described by Dreyer), flowers were present in November (Salter, 1944; Kumwenda et al., 2004). Flowering this late in the season sharply contrasts with most other CFR *Oxalis* species, which mainly flower during the wetter winter months only (May–July) (Dreyer et al., 2006). During 2005 the type locality of this species was visited on three separate occasions during October and November (according to its recorded flowering period) and not a single plant was located. The vegetation cover was much denser than in 2001 and the soil was very dry, with no evident seepage bands detected on the mountain slopes. Total rainfall for 2005 (140 mm) was also far below average for this region (average of 209 mm over 40 years) in contrast with 2001 during which the total rainfall was well-above the average (286 mm). To verify that the species does not vary its flowering period, and thus could have escaped detection during 2005, the type locality was revisited on various occasions during 2006 (June–November). Interestingly the site was burnt early in 2006, providing a similar, open habitat as had been recorded in 2001. Again not a single plant could be found on any one of the visits. In addition, the slope on which this species supposedly occurs is now heavily eroded. As in 2005, the total rainfall for 2006 (190

mm) was below average for the region. The fire early in 2006 was also followed by very early winter rain (92 mm during May). This differs from the pattern experienced in 2001 when most of the winter rainfall was recorded much later (105.5 mm during July) and for 2005 during which the winter rainfall was more evenly spread throughout the season (April–September).

O. oreophila has a short flowering period in May. This species is restricted to grass and Restionaceae dominated water seepage bands in the Pakhuis Pass Mountain. It occurs in a vegetation type that is best described as an ecotone of renosterveld and fynbos on seasonally wet shale bands running down the slopes of these sandstone mountains.

O. natans is an aquatic plant with a long, slender, flexuous stems and shortly petiolate leaves that float on the surface of the water. It has a restricted flowering period during September. The population studied in the Stellenbosch area (*O. natans* 1) occurs in a heavily disturbed lowland renosterveld vlei (seasonal marshland). In this specific population the small white flowers were confined to a stratum below the rest of the vegetation. A different white-flowered stratum was also present *ca.* 30 cm above the flowers of *O. natans*, and comprised of white-flowered species of *Onixotis*, *Ornithogalum* and *Spiloxene*. The population studied at Elandsberg Nature Reserve (*O. natans* 2) also occurs in a vlei habitat (vernal pool), but here it occurs in natural renosterveld. It is a large, healthy population with *O. natans* as the dominant species. Flowers in this population are much more exposed and seemingly subjected to less pollinator-competition than *O. natans* (1).

Both of the studied widespread species (*O. purpurea* and *O. tomentosa*) have wide distribution ranges in the Western Cape Province, and they mostly occur in very large populations. *O. purpurea* does not have such specific habitat requirements as the rare species, while *O. tomentosa* is mostly restricted to shale-derived clay soils in renosterveld. The population of *O. tomentosa* (1) studied at Darling occurs in fragmented renosterveld patches surrounded by cultivated lands. These patches mostly consist of plants of the same morph type. The populations studied at Saron (*O. tomentosa* 2) and Elandsberg (*O. tomentosa* 3) both represented much larger, unfragmented populations. All three populations of *O. purpurea* were growing in highly disturbed areas, especially *O. purpurea* (3) from Stellenbosch. The flowering period of *O. tomentosa* extends from April to June, while *O. purpurea* has an extended flowering period from April to October. In contrast to most of the rare species, these plants are more robust and they often produce many flowers per plant over a longer period.

The presence of potential pollinators was casually observed during population surveys, but no in-depth study into the pollination biology was undertaken. Most *Oxalis* species were visited by generalist pollinators, including honey bees (*Apis mellifera* ssp. *capensis*) that were observed visiting *O. comptonii*, *O. giftbergensis*, *O. oculifera*, *O. oreophila*, *O. tomentosa* and *O. purpurea*. A small fly (Diptera cf. *Empididae* sp.) was observed in the flowers of *O. oreophila* and *O. natans*. Small solitary bees (Hymenoptera cf. *Colletidae* sp.) quite commonly visited *O. giftbergensis*, *O. oreophila* and *O. tomentosa*. *O. oculifera* was visited by a butterfly species that also visited other purple flowers (e.g. Aizoaceae) in the region, while a scarab beetle (Melalanthinae) was found in flowers of *O. tomentosa*.

All of these potential pollinators were found to be more active and abundant in populations of the widespread species. No potential pollinators were observed associated with *O. amblyosepala* or *O. oligophylla*.

DISCUSSION

Results indicate that rarity is controlled or caused by different factors in the different *Oxalis* species studied and is often the result of a combination of several factors. Results are discussed under the main headings (Reproductive Biology and Ecology) as in the rest of the manuscript. Collective results of the rare species are discussed and compared throughout, to assess the expression of traits that may contribute to rarity of a particular species. This is followed by comparisons with traits of the widespread species, in order to identify the factors controlling rarity in *Oxalis*.

I. REPRODUCTIVE BIOLOGY

Fully tristylous populations (rare species)

O. natans and *O. amblyosepala* are completely self-incompatible and both populations are at isoplethic equilibrium (morph frequency ratio close to 1:1:1). In terms of morph morphology, *O. amblyosepala* is morphologically truly tristylous, as it displays three clearly distinct morph types (Fig. 3). Despite slight deviations in floral morph morphology in *O. natans*, which may be attributed to the diminutive size of the flowers (organs situated close together), both populations

of this species are fully tristylous and therefore dependant on pollinator and mate availability for sexual reproduction.

The breeding system of *O. amblyosepala* was studied in a small, isolated population in the Gifberg during 2005. Due to the lower rainfall during that year, the population was restricted and spread across a small number of isolated habitat patches (see Results, section Ecology). A fully expressed tristylous breeding system can be restrictive to sexual reproduction in small, isolated populations where the requirements of an effective pollen vector and equal availability of the three floral morph types are often not met (Brys et al., 2004). These impediments may be operational in this natural population of *O. amblyosepala*, as this completely self-incompatible population displays statistically significant lower levels of natural seed production compared to seed production following artificial legitimate pollinations. As this population is at isoplethic equilibrium, morph availability would not restrict sexual success. It can thus be deduced that the limited natural seed production in this population results from reduced natural pollination success. It is well-known that small population sizes often lead to disrupted biological interactions (Luijten et al., 2000; Hooftman et al., 2003).

The highly disturbed population of *O. natans* (*O. natans* 1) was also at isoplethic equilibrium, which implies that morph availability was not responsible for the extremely low natural seed set observed (Table 5). Therefore, reduced insect-pollination success also seems to be limiting seed production in this completely self-incompatible population. In this case failed pollinator success could be the result of the flowers not being visible or accessible to pollinators, as all of the flowers are presented in a stratum below an upper stratum (ca. 30 cm above the *Oxalis* flowers) composed of a combination of other white-flowered plant species. The problems in this population are further exacerbated since even artificial legitimate pollinations produced almost no seeds. These low levels of seed production could rather also be a result of low fecundity as a consequence of inbreeding depression in small populations (Hooftman et al., 2003). This explains the lack of significant difference in seed production following selfing vs. legitimate cross-pollinations which is usually expected for highly self-compatible populations. This completely self-incompatible population, which possibly experience both pollinator stress and reduced fecundity, therefore has to rely almost entirely on vegetative reproduction for persistence. Results indeed confirmed very high levels of clonality within this population (Table 7). Burne et al. (2003) suggested that high levels of clonality may be related to the loss of capacity to reproduce sexually. It appears that this population of *O. natans* has lost its capacity

to produce seeds due to the restrictions associated with tristylly, and that it has currently become almost completely dependant on vegetative reproduction for survival. Alternatively the population might simply have an extremely low fecundity as a result of inbreeding depression, so that asexual reproduction offers the only way to persist. This would need to be confirmed by population genetic analyses.

In contrast *O. natans* (2) occurs in a natural habitat in which the potentially pollination-inhibiting white-flowered stratum above the *O. natans* flowers is absent. This population is also completely self-incompatible, but showed much higher levels of natural seed production compared to *O. natans* (1) (Table 5) and even controlled legitimate pollinations resulted in a high percentage of seed production (Table 4). The difference in natural seed production vs. controlled legitimate pollinations was not significant, implying that seed production in the wild is very close to the full potential of this population. This tristylous population, which displays only moderate levels of clonality (Table 7), is therefore not affected by possible limitation of tristylly, pollinator competition and/or low fecundity as in the case of *O. natans* (1).

The *O. gifbergensis* and *O. oreophila* populations also appear to be fully tristylous when all their attributes are combined, except for a very slight slippage in their self-incompatibility that results in seed production following self-pollination (Table 3). These low levels of selfing were, however, not significant and the ISI is also much lower than the arbitrary proposed number of 0.20 required to characterize a population as partially self-compatible (Table 5). Both species showed noticeably higher levels of natural seed production than any of the previously discussed species (Table 5). It thus appears as if, as in the *O. natans* (2) population, tristylly is less inhibiting and less of a survival threat in populations of these two species. The *O. oreophila* population displayed moderate levels of clonality, as was indeed the case for all of the rare species studied, except for *O. natans* (1) and *O. gifbergensis* (Table 7). *O. gifbergensis*, however, showed very low levels of clonality, implying that sexual reproduction is efficient and adequate, and there appears to be no immediate dependence on clonal propagation for survival.

Alterations in tristylly expression (rare species)

O. comptonii and *O. oculifera* show a very interesting contrast to the patterns discussed above. Both species produced an exceptionally high percentage of seeds following self-pollinations and for both populations the L morph type displayed a significantly higher percentage seed production following selfing than the other morph types. The total ISI is also high enough to

characterize *O. comptonii* as partially self-compatible and the ISI of *O. oculifera* is also higher than in the rest of the rare populations studied (Table 5). Populations of both of these species also have morph frequency ratios that deviate significantly from the expected 1:1:1 ratio and they show morphological forms that differ from the morphology in normal, fully tristylous species (Fig. 3). Morph frequencies have frequently been found to deviate from isoplethic equilibrium in small natural populations and can be ascribed to genetic drift, founder effects or population bottlenecks (Eckert and Barrett, 1992; Byers, 1995). The non-isoplethic population structure of these populations suggests that levels of natural seed production should be low due to unavailability of morph types (Byers 1995). In contrast, though, there were no significant differences between natural seed production and seed production following controlled legitimate cross-pollinations.

The observed breakdown in tristily thus appears to be beneficial to these species, as it enables seed production regardless of pollinator availability and/or the population structure (morph representation). In such populations or species, reproductive assurance may be more important than the genetic variability attained by cross-pollination (Barrett 1985). If, however, the habitat becomes threatened to the extent that it leads to a decline in population size, even selfing would not be able to prevent extinction because of threats associated with small population sizes. Selfing can therefore be viewed only as a short-term survival strategy for rare plant species, which may only postpone extinction. On the other hand, self-compatible populations may still undergo chance cross-pollinations if pollinators are still available. This seems to be the case in both *O. comptonii* and *O. oculifera*, as potential pollinators were observed to visit these species in their natural habitat. Such chance legitimate pollinations would increase genetic variability, which would lead to an increase in the ability of the population to persist. These species are potentially cross-pollinating, while the potential for selfing may simply enable them to overcome the limitations of unequal morph availability and unfavorable conditions for pollinators. This constitutes a relatively viable scenario for rare *Oxalis* species.

From the limited results available for *O. oligophylla* we can deduce that the population is characterized by an extremely low density, moderate level of clonality and although this population displayed a healthy population structure close to isoplethic equilibrium, it showed markedly aberrant morph morphologies. Studies by Van Rossum et al. (2004) showed that pollinators moved more frequently between individuals of *Primula* spp. in populations with high densities, while they spent more time on the same plant in populations with low densities. They

concluded that this may favor selfing in sparse populations. Studies by Franceschinelli and Bawa (2000) also showed a positive correlation between plant density and out-crossing rates in *Helicteres brevispira* ST. Hill. Low densities in populations in combination with moderate clonal growth could alternatively also enhance movement of pollinators between different morph types as these plants have only 2–3 flowers/plant. The low densities and morphological deviations observed in this species thus merit further investigations into the expression of self-incompatibility and natural seed production in *O. oligophylla*.

Populations of the widespread species

The populations of the widespread *O. purpurea* varied in their expression of self-incompatibility. Controlled self-pollinations yielded some seeds in all three populations (Table 3). *O. purpurea* (1) appears to be fully tristylous in terms of the population structure and morph morphology, and displayed insignificant levels of self-compatibility. Similar low levels of selfing in *O. purpurea* (2), however, are accompanied by unequal morph representation and some deviation in the morph morphology from truly tristylous populations. In both of these populations seed production following controlled legitimate cross-pollination was exceptionally high when compared to the rare species (Table 4). Populations of the widespread *O. purpurea* are potentially much more fecund than populations of the rare species, which have a lower potential seed production as seed production is expected to be at a maximum in controlled legitimate pollinations. This may be the result of inbreeding depression and/or lower genetic variability due to small population sizes and isolation of rare species (Pullin, 2002; Hooftman et al., 2003). Such negative factors appear to be strongly expressed in *O. natans* (1), in which legitimate seed production is incredibly low.

Although levels of natural seed production were also very high for these populations of *O. purpurea*, it was significantly lower than those following legitimate cross-pollinations. This could be due to the extremely high potential seed production in this endospermous species, where capsules can contain many ovaries per locule (Salter, 1944) (up to 50 seeds per capsule observed for *O. purpurea*). The percentage natural seed production, although lower than this potential, still appears to be very high and was certainly much higher than in most of the rare species studied (Table 5).

O. purpurea (3) contains only plants with the L floral morph type. The remarkable morphological alterations seem to occur in order to ensure self-pollination (Fig. 4) in this

completely self-compatible population ($ISI > 0.8-1$). It is located on the eastern periphery of the distribution range of *O. purpurea* and was possibly established by founder events during which all the genotypes were not present to establish a population at isoplethic equilibrium (Ornduff, 1964). As only one morph type is present, one would expect that this population can only propagate clonally. In this population, however, it appears as if the tristylous-associated limitations to sexual recruitment have been overcome through a complete breakdown in the incompatibility system. Seed production is thus ensured regardless of the unavailability of compatible morph types. The success of self-pollination is further supported by the high percentage of naturally produced seeds, despite the fact that legitimate cross-pollination is impossible. The average number of seeds per capsule produced naturally in *O. purpurea* (3) is even higher than in the *O. purpurea* (1) and (2) populations, which indicates that selfing is very successful within this population (Table 5).

Natural seed production was very low for all the studied populations of the widespread *O. tomentosa* (Table 5). From the available results for *O. tomentosa* (no experimental self- and cross-pollinations could be conducted), *O. tomentosa* (2) and (3) exhibit characteristics that suggest that the tristylous breeding system is still intact (isoplethic equilibrium and typical tristylous floral morphology). It would therefore be extremely interesting to compare the low levels of natural seed production observed for these two populations (Table 5) to controlled legitimate cross-pollinations. For the morphologically tristylous *O. tomentosa* (1) no seed production was observed in the wild and this fragmented population also displayed a morph frequency ratio that deviates significantly from isoplethic equilibrium (1:1:1). It is therefore assumed that sexual reproduction is restricted by the unavailability of all the morph types in fragmented patches. This could correlate to pollinator availability and activity within and between these fragmented patches. Unlike the monomorphic population *O. purpurea* (3), this morphologically tristylous population of *O. tomentosa* appears not to be able to produce seed through self-pollination, a situation similar to what was found in *O. natans* (1). Both *O. tomentosa* (1) and *O. natans* (1) appear to be entirely dependant on vegetative reproduction for survival.

Vegetative reproduction

The onset and duration of flowering in southern African *Oxalis* species are influenced by both the onset and quantity of winter rainfall and the average daily temperatures (Dreyer et al., 2006). In drier years, less successful seed production due to reduced flowering could be compensated

for by vegetative reproduction via bulbil formation (characteristic of all the southern African members of *Oxalis*) (Salter, 1944). Over the longer term very high levels of clonality would, however, lead to reduced genetic diversity and could also reduce fecundity. Ellstrand and Antonovics (1985) found that populations of asexually derived individuals had lower survivorship and fecundity than populations of sexually derived individuals. The level of clonality appears to be very variable within and between *Oxalis* species. The very low level of clonal growth observed in the *O. giftbergensis* population may increase if conditions for seed production should become unfavorable. This seems to have taken place in *O. natans* (1) and *O. tomentosa* (1), both representing populations that are experiencing very unfavorable conditions for sexual reproduction. Both of these populations display higher levels of clonality than the other populations of *O. natans* and *O. tomentosa* that are less restricted (Table 7). Extremely high levels of clonal propagation, as was evident for the widespread species, may aid or facilitate the establishment of new populations, especially in species such as *O. purpurea* with very flexible habitat requirements.

Extensive clonal propagation could be a further threat to the survival of completely self-incompatible rare species, as it could result in skewed morph frequency ratios (Ornduff, 1964; Barrett, 1977) and thus reduce the possibility for natural seed formation. In *O. natans* (1) and *O. amblyosepala*, for example, natural seed production is already extremely low, which suggests that increased levels of clonality could be detrimental in such cases. For self-compatible populations (*O. purpurea* 1, *O. comptonii* and *O. oculifera*), skewing of morph frequency ratios does not appear to affect seed production negatively. Extensive clonal growth, as was observed in *O. purpurea* (3), could increase the number of potential seed-producing individuals, and thus positively contribute to increased population sizes.

II. ECOLOGY

The highly specific habitat requirements of many of the rare species probably confine them to small, isolated populations within highly specific habitats. They are unable to increase their population sizes due to the unavailability of suitable habitat and are under severe threat should their available habitat be disrupted or fragmented. Examples of such species include *O. amblyosepala*, *O. comptonii*, *O. oculifera*, *O. oreophila* and *O. hygrophila*, all of which appear to be naturally rare, new endemics. Others might have been formerly widely distributed and are now only confined to habitat relicts e.g. *O. giftbergensis* (restricted to refugium arid fynbos) and

O. natans (fragmented by urbanization). In the next 50 years, a 0.5°–1.0° C increase in temperature as well as a 25% decrease in annual rainfall is predicted for the Cape Region (Rutherford et al., 1999). This change in climate is expected to have a range of consequences for biodiversity and a dramatic impact on the Cape flora (Bond, 1997). This is a major threat to most of the rare *Oxalis* species confined to seasonally waterlogged or aquatic habitats in the Western Cape. The potentially severe negative impacts of climate change on such species can already be observed in *O. hygrophila*, a species that is restricted to water-logged seepage bands, of which no plants could be found during 2005 or 2006. Other species (especially the Gifberg endemics confined to seasonally waterlogged habitat patches), also showed remarkable variation in the number and sizes of populations, which coincided with variable rainfall patterns observed over the study years.

As most *Oxalis* species are winter-flowering, it is rather unusual for *O. hygrophila* to flower in November. The benefit of such a strategy may be to escape competition for limited pollinators during the colder winter months when most other *Oxalis* species flower. It appears, however, to be a risky strategy for a species such as *O. hygrophila* that is confined to seasonally waterlogged habitats, as November is not within the rainy season in the CFR. Extensive rainfall early in the 2006 season resulted in a very good flowering season for the whole Cape Region, including the Gifberg and Pakhuis Pass study areas. This early rainfall was, however, ineffective for late flowering species such as *O. hygrophila*. It appears that this species is not able to shift its flowering period towards earlier winter rainfall months, and that above-average rainfall later in the season provided the suitable, waterlogged habitat in which *O. hygrophila* was collected in November 2001. *O. oreophila*, which occurs in a similar waterlogged habitat to *O. hygrophila* in the Pakhuis Pass was, however, present in 2005 and 2006. The difference is that the latter species flowers during the winter months (May to June), in the middle of the rainy season. With lower rainfall in 2005 and 2006, the habitat of the latter species was therefore still waterlogged during its flowering period.

In addition, the locality of the known *O. hygrophila* population is now heavily eroded. This erosion could be a result of a lack of vegetation cover after the fire early in 2006 followed by heavy winter rainfall that commenced early in May. These events obviously negatively impacted this species, and it is critically important to monitor this locality if rainfall patterns similar to 2001 are observed in future to verify whether this species does, in fact, still exist.

The lower potential for seed production observed in rare species as compared to widespread species could result from the negative effects of small population sizes, as was discussed previously. The very low density of *O. oligophylla* may, for example, be a major threat where range fragmentation will result in very small populations, with only a few individuals, unable to persist as discussed by Primack (1993). Many of the rare species studied currently exist in only a few isolated populations (*O. amblyosepala*, *O. comptonii*, *O. oculifera*, and *O. oreophila*). A loss of a single one of these populations will have a very negative impact on the future survival of the species, as a large proportion of the total genetic variation will be lost. This is obviously extremely precarious in species currently confined to a single known population, such as *O. hygrophila*.

The widespread species display obvious shared ecological characters that differ from the rare species. They lack specific habitat requirements and have a weedy nature, especially in *O. purpurea*. This provides the widespread species with more available habitat and larger potential to increase their population sizes. Large population sizes, together with larger flowers and more flowers per plant, may cause these species to be much more visible to pollinators. *O. purpurea* also has the benefit of an extended flowering period and different flower colours present in different parts of its natural distribution range. This may be significant in terms of attracting different pollinators in different parts of its range. The ability for extensive vegetative reproduction via bulbil formation in the widespread species may also facilitate the establishment of new populations. Extensive clonal growth in combination with excessive seed production, serves as a double strategy to ensure large population sizes of such species. The variation in the self-incompatibility system observed in different populations of *O. purpurea* could be viewed as advantageous when a particular population is limited by the requirements of this complex breeding system, especially in disturbed habitats. The two widespread species also develop more robust plants that appear to be more resistant against physical damage or destruction than the rare species. It thus seems as if a combination of various biological attributes of *O. purpurea* and *O. tomentosa* ensure their wide distribution and abundance.

Although *O. tomentosa* is widespread in the western Cape and not considered to be rare, it appears that many of its populations might be endangered due to fragmentation as observed for *O. tomentosa* (1). This population is fragmented into smaller patches that do not always contain all three floral morph types. It does not produce any seed in the wild, and produces very few flowers compared to the larger and more natural *O. tomentosa* populations studied. This

widespread species (restricted to renosterveld ridges) is subjected to habitat fragmentation and disturbance within its natural distribution range, as large portions of renosterveld has been lost to agriculture (Kemper et al., 1998). Although this species is common in the Western Cape, it could possibly have displayed a much larger, continuous population in the past. This agrees with the suggestions by Van Rossum et al. (2004) who argue that commonness is not always a guarantee for long term survival, especially in fragmented habitats. It is therefore of great importance to preserve the habitats of not only rare species, but also widespread species that displays habitat specificity. This may be attained through habitat refuges on road verges and steep slopes unsuitable for agriculture, which may function as natural corridors between patches in fragmented landscapes (Kwak et al., 1998).

CONCLUSIONS

Tristyly expression is rather unstable and extremely variable among the *Oxalis* species studied. The reproductive success of three of the studied populations appears to be limited by the tristylous breeding system, namely a natural population of the rare species *O. amblyosepala*, the disturbed population of the rare *O. natans* (1) and the fragmented population of the widespread *O. tomentosa* (1). For *O. natans* (1) and *O. tomentosa* (1), reduced reproductive success could also be a result of low fecundity in small/disturbed populations. The reproductive success of *O. natans* (2), *O. giftbergensis* and *O. oreophila* is not restricted by the tristylous breeding system and they are not under immediate threat provided that their habitats can be preserved. Other populations overcome possible limitations to sexual reproduction by tristylous breakdown/relaxed self-incompatibility (*O. comptonii*, *O. oculifera* and *O. purpurea*), while still retaining the potential to out-cross if chance cross-pollinations are possible.

Most of the rare/endangered species studied are limited by their highly specific habitat requirements. Species confined to seasonally waterlogged habitats are of the most urgent concern, based on the proposed implications of climate change and the visible reproductive variability of these species under variable rainfall patterns.

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APPENDIX 1

Details of field data including the geographical localities, habitat attributes and distributions of each of the studied populations.

Species		<i>O. amblyosepala</i>	<i>O. comptonii</i>	<i>O. giftbergensis</i>	<i>O. natans</i> (1)	<i>O. natans</i> (2)	<i>O. oculifera</i>	<i>O. oligophylla</i>
Location	Region	Western Cape	Western Cape	Western Cape	Western Cape	Western Cape	Western Cape	Western Cape
	Major	Vanrhynsdorp	Vanrhynsdorp	Vanrhynsdorp	Stellenbosch	Bo-Hermon	Vanrhynsdorp	Vanrhynsdorp
	Minor	Gifberg summit	Gifberg summit	Gifberg	Chelkim Farm	Elandsberg Nature Reserve	Gifberg summit	Gifberg
	Precise	On Ouberg turnoff at Nuwelskloof-Gifberg rusoord fork	On Ouberg tunroff, 1km along at first turnoff left to Sewefontein	Surroundings at Ouberg turnoff	Firgrove/Macasar Road, Raithby turnoff	Elanskloof river crosses into channel among old lands	± 700m along Ouberg tunoff, 400m along road to Vermaak	5 km from Wiedouw Boerdery turnoff along Gifberg pass
	Grid reference	S 31° 46.678'	S 31° 46.574'	S 31° 46.573'	S 34° 01.097'	S 33° 25.336'	S 31° 46.912'	S 31° 45.922'
		E 18° 45.960'	E 18° 46.519'	E 18° 45.823'	E 18° 48.327'	E 19° 01.673'	E 18° 46.011'	E 18° 46.164'
Altitude (m)	588	621	593	-	-	576	498	
Habitat	Vegetation	Fynbos	Fynbos	Arid Fynbos	Degraded lowland renosterveld	Renosterveld	Fynbos	Fynbos
	Substrate	Shallow pockets of soil on flat rocks	Lense of soil and moss on solid rock	Soil and gravel	Waterlogged soil	Waterlogged soil	Lense of soil and moss on solid rock	In cracks and soil on and between rocks on stepped cliffs
	Soil type	Sand and clay	Fine clay and sand	Sandy clay	Sand and clay	Sand on clay base	Fine clay	Sand
	Lithology	Sandstone	Sandstone	Sandstone	Shale base	Shale base	Sandstone	Sandstone
	Moisture regime	Seasonally waterlogged crevices/pockets of soil	Seasonally waterlogged	Dry	Aquatic	Aquatic	Seasonally waterlogged	Seasonally moist cliffs
	Exposure	Full sun/ semi shade under shrubs	Full sun	In shade of shrubs, flowers in sun.	Full sun	Full sun	Full sun	Shade of cliffs
	Aspect			S				S
	Slope	Level	Level	Level	Level	Level	Level	Steep
	Disturbance	Natural	Natural	Natural	Disturbed	Natural	Natural	Natural
Description of habitat	Restionaceae dominated flat plane with seasonally waterlogged pockets of soil on flat rocks in open sun. Surrounded by denser Fynbos.	Many small seasonally waterlogged habitat patches. Thin layer of soil and moss on solid rock. In pathway of stream. Natural and vulnerable, distinct habitat.	Refugium Arid Fynbos all over mountain with an occasional emergant <i>Protea</i> species. Dense cover with open areas in between.	Heavily disturbed seasonal vlei in cultivated land. Plants floating on water beneath rest of vegetation.	Natural seasonal vlei area in Renosterveld. Plants floating on open water.	Many small seasonally waterlogged habitat patches. Thin layer of soil and moss on solid rock. In pathway of stream. Natural and vulnerable, distinct habitat.	Grow in cracks on and between rocks on stepped cliffs. High on escarpment of mountain.	

Species		<i>O. amblyosepala</i>	<i>O. comptonii</i>	<i>O. giftbergensis</i>	<i>O. natans</i> (1)	<i>O. natans</i> (2)	<i>O. oculifera</i>	<i>O. oligophylla</i>
Habitat	Description of vegetation	Asteraceae spp. (various species), Mesembryanthemaceae cf. <i>Ruschia</i> sp., Restionaceae (dominant), Thymelaeaceae, <i>Arctopus</i> sp., <i>Crossyne</i> sp., <i>Drosera</i> sp., <i>Heliophila</i> sp. (small white flowers), <i>Spiloxene</i> sp. (yellow). <i>Oxalis flava</i> , <i>O. giftbergensis</i> , <i>O. obtusa</i> , <i>O. pes-caprae</i> , <i>O. purpurea</i> ; also rich in moss and lichens.	Growing amongst moss, ferns, lichens, Mesembryanthemaceae cf. <i>Ruschia</i> , <i>Babiana</i> sp., <i>Drosera</i> sp., <i>Heliophila</i> sp., <i>Spiloxene</i> sp. (purple mimicry with <i>Oxalis</i> ??). <i>Oxalis obtusa</i> , <i>O. oculifera</i> , <i>O. purpurea</i> . Interspersed by taller Fynbos elements on edges in deeper soil, e.g. <i>Metalasia</i> sp., <i>Pelargonium</i> sp., <i>Restio</i> spp., <i>Stoebe</i> sp.	Poaceae, <i>Felicia</i> spp. (dominant), <i>Ruschia</i> spp. (purple - in understory), <i>Anthospermum</i> sp., <i>Cliffortia</i> sp., <i>Crossyne</i> sp., <i>Eriocephalus</i> sp., <i>Euryops</i> sp., <i>Metalasia</i> sp., <i>Muraltia</i> sp., <i>Nylandtia</i> sp., <i>Protea</i> spp. (occasional). <i>Oxalis obtusa</i> , <i>O. purpurea</i> , <i>O. flava</i>	Dominant white-flowered stratum (<i>Onixotis</i> sp., <i>Ornithogalum</i> sp., <i>Spiloxene</i> sp.) ca. 30 cm above <i>O. natans</i> . Asteraceae, Cyperaceae. <i>Disa</i> sp., <i>Drosera</i> sp., <i>Morea</i> sp., <i>Sebaea</i> sp., <i>Briza maxima</i> , <i>B. minima</i> , <i>Oxalis glabra</i> , <i>O. nidulans</i> , <i>O. pes-caprae</i> , <i>O. purpurea</i> , <i>Zantheschia aethiopica</i> . <i>Echium</i> sp. (invasive weed).	<i>O. natans</i> dominates in water. <i>Crasula natans</i> (floating), <i>Drosera</i> sp., <i>Moraea tripetala</i> , <i>Albuca</i> sp., <i>Lachenalia</i> sp., <i>Ornithogalum</i> sp., <i>Oxalis multicaulis</i> and <i>O. ghoniorhisa</i> occur on dryer patches.	Growing amongst moss, ferns, lichens, Mesembryanthemaceae cf. <i>Ruschia</i> , <i>Babiana</i> sp., <i>Drosera</i> sp., <i>Heliophila</i> sp., <i>Spiloxene</i> sp. (purple mimicry with <i>Oxalis</i> ??). <i>Oxalis comptonii</i> , <i>O. obtusa</i> , <i>O. purpurea</i> . Interspersed by taller Fynbos elements on edges in deeper soil, e.g. <i>Metalasia</i> sp., <i>Pelargonium</i> sp., <i>Restio</i> spp., <i>Stoebe</i> sp.	Poaceae, Restionaceae dominated (<i>Restio</i> spp., <i>Elegia</i> spp.). <i>Cliffortia</i> sp., <i>Dodonea</i> sp., <i>Erica</i> sp. (red flower), <i>Metalasia</i> sp., <i>Silene</i> sp.; <i>Pelargonium glutinosum</i> , <i>Protea nitida</i> .
Phenology	Flowering starts May. Full flowering June-July. Bud formation still occurs in July. Seeding period mostly July.	Flowering starts May. Full flower in June. Seeding period July, with bud formation and flowering much reduced.	Full flower May. June, mostly seed.	Flowering September. Hardly any seeding observed.	Flowering September-October.	Flowering starts May. Full flowering June-July. Bud formation still occurs in July. Seeding period mostly July.	Flowering May-June.	
Plant features	Dwarf plant, less than 5 cm tall. Very shallow, small bulb. White flower with outer margins of petals pink. Up to 5 flowers/plant.	Dwarf plant, 5 cm tall. Flowers white, tube greenish yellow. Up to 7 flowers/plant.	Grow underneath and between shrubs. Bright pink flowers emerging between vegetation, shallow bulbs protected from sun. Plants up to 20 cm tall. 3-8 flowers/plant.	White flowers, floating leaves. Long, slender, flexuous stem, 2-5 flowers/plant.	White flowers, floating leaves. Long, slender, flexuous stem, 2-5 flowers/plant.	Dwarf plant, 2 cm tall. Violet pink flowers with white ring in throat and wine-red tube. 2-5 flowers/plant.	Very slender plants; 2 leaves per plant with long, linear, slender leaflets; flower white, 1-3 flowers/plant.	

Species		<i>O. amblyosepala</i>	<i>O. comptonii</i>	<i>O. giftbergensis</i>	<i>O. natans</i> (1)	<i>O. natans</i> (2)	<i>O. oculifera</i>	<i>O. oligophylla</i>
Pollinators		No pollinators observed	Hymenoptera: <i>Apis mellifera</i> ssp. <i>capensis</i> (honey bee)	Hymenoptera: <i>Apis mellifera</i> ssp. <i>capensis</i> (honey bee) cf. Colletidae sp. (solitary bee)	Diptera: cf. Empididae sp.	Diptera: cf. Empididae sp.	Hymenoptera: <i>Apis mellifera</i> ssp. <i>capensis</i> , Lepidoptera visiting plants with similar looking flowers	No pollinators observed
Population	Density	30 plants/m ² High local density in small patches	50 plants/m ² High local density in small patches	5 plants/m ² Plants well-spaced	15 plants/m ²	20 plants/m ²	>500 plants/m ² High local density in small patches .	1 plant/10m ² Very low density.
	Size and Distribution	<i>Restio</i> - dominated flat plane (60m X 20m). Several localities with similar habitat features on Giftberg, varying according to rainfall.	Small patches (1-10m ²) restricted to pathway of small stream. About five patches in area of 50 m X 50 m. Possibly more sub-populations in similar habitat patches on Giftberg.	Locally abundant on mountain which can be viewed as an island of Fynbos surrounded by Succulent Karoo. Large parches (100m X 100m).	Locally abunded in vlei area. Large patch (100 m X 50 m).	Locally abunded in vlei area along river. Large patch (100 m X 6 m).	Small patches (1-10m ²) restricted to pathway of small stream. About 25 small patches in area of 200m X 300m. Possibly more sub- populations in similar habitat patches on Giftberg	Widely distributed high on escarpment in area of 200 m X 50 m.

Species		<i>O. oreophila</i>	<i>O. purpurea</i> (1)	<i>O. purpurea</i> (2)	<i>O. purpurea</i> (3)	<i>O. tomentosa</i> (1)	<i>O. tomentosa</i> (2)	<i>O. tomentosa</i> (3)
Location	Region	Western Cape	Western Cape	Western Cape	Southern Cape	Western Cape	Western Cape	Western Cape
	Major	Clanwilliam	Stellenbosch	Citrusdal	George	Darling	Saron	Bo-Hermon
	Minor	Pakhuis Pass	J. S. Marais Park	Piekenierskloof Pass (N7)	On N9 George to Uniondale	The Towers Farm	Saron, R44	Elandsberg Nature Reserve
	Precise	Near summit ± 3.7 km from start of tar road	On lawn	Southern end of pass	60 km before Uniondale	Near gate on way to tower	500 m along tunroff from R44 to Saron	Near end of Tortois road
	Grid reference	S 32° 08.932' E 19° 01.761'	S 33° 56.020' E 18° 52.418'	S 32° 38.921' E 18° 57.063'	S 33° 46.028' E 22° 48.406'	S 33° 25.098' E 18° 23.115'	S 33° 11.305' E 18° 59.060'	S 33° 25.607' E 19° 02.762'
	Altitude (m)	910	-	374	649	295	76	-
	Habitat	Vegetation	Ecotone Renosterveld & Fynbos	Grass lawn	Ecotone Renosterveld & Succulent Karoo	Ecotone Renosterveld & Karoo (degraded)	Renosterveld	Degraded Renosterveld
Substrate		Soil and gravel	Soil	Soil and gravel	Soil and gravel	Soil and gravel	Soil and gravel	Soil and gravel
Soil type		Clay	Sand	Sand	Sandy loam	Sand and clay	Clay	Clay
Lithology		Shale bands	Sandstone	Shale		Shale	Shale	Shale
Moisture regime		Natural water seepage band, seasonally wet	Well-drained	Well-drained	Dry	Poorly drained	Poorly drained	Poorly drained
Exposure		Full sun/ underneath shrubs	Full sun	Full sun	Full sun	Full sun	Full sun	Full sun
Aspect		S		S		W		
Slope		Moderate	Level	Level to moderate	Level	Moderate to steep	Level	Level
Disturbance		Natural	Disturbed	Disturbed	Disturbed	Fragmented	Disturbed	Natural
Description of habitat	Earthworm activity. Grass and restio dominated seepage bands (seasonally waterlogged). Bands of shale running down a sandstone mountain. Resently burned patch.	Highly disturbed on grass lawn.	Highly disturbed on side of road.	Highly disturbed on side of road.	Highly disturbed on side of road.	Natural Renosterveld fragments surrounded by cultivated land.	On side of road and in cultivated land.	Renosterveld ridge in natural vegetation.

Species		<i>O. oreophila</i>	<i>O. purpurea</i> (1)	<i>O. purpurea</i> (2)	<i>O. purpurea</i> (3)	<i>O. tomentosa</i> (1)	<i>O. tomentosa</i> (2)	<i>O. tomentosa</i> (3)
Habitat	Description of vegetation	Asteraceae (annual and perennial spp.), Poaceae, Thymelaeaceae, <i>Arctopus</i> sp., <i>Cliffortia</i> sp., <i>Eriosephalus</i> sp., <i>Restio</i> spp., <i>Stoebe</i> sp., <i>Elytropappus rhinocerotis</i> (renosterbos).	No natural vegetation.	Euphorbiaceae, <i>Elytropappus rhinocerotis</i> , <i>Galenia africana</i> , <i>Eucalyptus</i> sp., <i>Microlema</i> sp., <i>Ruschia</i> sp., <i>Oxalis viscosa</i> , <i>O. pes-caprae</i> , several geophytes.	Poaceae, Mesembryanthemaceae, <i>Atriplex semibocata</i> , <i>Arctopus</i> sp., <i>Oxalis caprina</i> , <i>O. hirta</i> , <i>O. obtusa</i> , <i>O. punctata</i> .	<i>Bulbine annua</i> , <i>Elytropappus rhinocerotis</i> , <i>Eriosephalus</i> sp., <i>Haemanthus coccineus</i> , <i>Oxalis hirta</i> , <i>O. monophila</i> , <i>O. pes-caprae</i> , several geophytes.	Poaceae spp. in cultivated land. <i>O. purpurea</i> , <i>O. pes-caprae</i> .	<i>Elytropappus rhinocerotis</i> , <i>Empodium plicatum</i> , <i>Restio</i> spp., <i>Oxalis glabra</i> , <i>O. pes-caprae</i> , <i>O. purpurea</i> .
Phenology	Full flower in May. Much reduced flowering in June. Seeding May-June.	Flowering April-October. Buds and seeds through flowering period.	Flowering April-October. Buds and seeds through flowering period.	Flowering April 2005. 2006 Flowering started May.	Flowering April to May.	Flowering April to June.	Flowering April to June.	
Plant features	White flowers with red outer margin on petals. Plants up to 15 cm tall. 1-3 flowers/plant.	Large, bright pink flowers with yellow tubes. Robust plants up to 15 cm tall. Up to 10 flowers/plant.	Large, white flowers with yellow tubes. Robust plants up to 15 cm tall. 5-16 flowers/plant.	Light pink or white flowers with yellow tubes. Up to 6 flowers/plant.	Large white flowers. Robust plants. Leaves subrosulate, hairy with 10-20 leaflets. Plants up to 12 cm tall. Up to 7 flowers per plant.	Large white flowers. Robust plants. Leaves subrosulate, hairy with 10-20 leaflets. Plants up to 12 cm tall. Up to 7 flowers per plant.	Large white flowers. Robust plants. Leaves subrosulate, hairy with 10-20 leaflets. Plants up to 12 cm tall. Up to 7 flowers per plant.	

Species		<i>O. oreophila</i>	<i>O. purpurea</i> (1)	<i>O. purpurea</i> (2)	<i>O. purpurea</i> (3)	<i>O. tomentosa</i> (1)	<i>O. tomentosa</i> (2)	<i>O. tomentosa</i> (3)
Pollinators		Diptera: cf. <i>Empididae</i> sp.) Hymenoptera: cf. <i>Colletidae</i> sp. Honey bees were also observed visiting most flowers in area	Hymenoptera: <i>Apis mellifera</i> ssp. <i>capensis</i>	Hymenoptera: <i>Apis mellifera</i> ssp. <i>capensis</i>	Hymenoptera: <i>Apis mellifera</i> ssp. <i>capensis</i>	Hymenoptera: <i>Apis mellifera</i> ssp. <i>capensis</i> . cf. <i>Colletidae</i> spp. also observed visiting other <i>Oxalis</i> spp. in area	Hymenoptera: <i>Apis mellifera</i> ssp. <i>capensis</i>	Hymenoptera: <i>Apis mellifera</i> ssp. <i>capensis</i> cf. <i>Colletidae</i> spp. and <i>Melanthinae</i> sp. observed inside flower
Population	Density	13 plants/m ² Plants well-spaced.	20 plants/m ² Clumped distribution.	15 plants/m ² Clumped distribution.	5 plants/m ² Clumped distribution.	5 plants/m ² within patches.	15 plants/m ² within patches.	20 plants/m ²
	Size and Distribution	Restricted to patches on shale bands in Pakhuis Pass (50m X 50m patch).	Large, dense population (50m X 30m). Clumps of the same morph type.	Large, dense population (50m X 10m). Clumps of the same morph type.	3m X 30m Only plants of the Long morph type.	Fragmented population in area of 40m X 100m. Clumps of plants of the same morph type within fragments.	Patchy distribution in area of 200m X 100m. Clumps of plants of the same morph type.	200m X 25m Clumps of plants of the same morph type.

CHAPTER 3

Phylogeographic patterns in *Oxalis* (Oxalidaceae): a tale of rarity, recent divergence and a widespread congener in the Cape Floristic Region**Abstract**

Oxalis L. is the largest and most diverse genus in the family Oxalidaceae. Within southern Africa, *Oxalis* is represented by ca. 270 taxa, the majority occurring in the Cape Floristic Region. Although many of the species are widespread, ca. 25% are considered rare. The aim of this paper is to assess the degree of genetic differentiation between two rare and highly localized species (*O. hygrophila* Dreyer and *O. oligophylla* Salter) and the more widespread *O. tomentosa* L.f. For comparative purposes, we also include *O. purpurea* L., one of the most widely distributed species in South Africa. Chloroplast sequences of the *trnH/psbA* spacer revealed low genetic diversity for *O. oligophylla* and *O. tomentosa* compared to the widespread *O. purpurea*. High genetic diversity in *O. purpurea* might, in combination with other ecological and reproductive factors, account for the success of this species. In contrast, low variation in *O. oligophylla* and *O. tomentosa* might contribute to their rarity. The latter two species were not monophyletic and shared a haplotype. Coalescent modeling revealed low levels of gene flow (< 1 migrant per generation) between them, and we argue that the genetic pattern is the result of the retention of ancestral polymorphism following a recent divergence.

Keywords: chloroplast intergenic spacer *trnH-psbA*, coalescent modelling, gene flow, *Oxalis*, population structure

INTRODUCTION

Oxalis L., consisting of almost 900 species, is the largest and most diverse genus in the family Oxalidaceae. It has a cosmopolitan distribution with two centres of diversity, namely southern Africa and South-Central America (Denton, 1973; Weller, 1992). This dicotyledonous genus is characterized by a variety of habits including procumbent herbs (Lourteig, 1979), geophytes with true bulbs (Salter, 1944) or tubers (Emshwiller & Doyle, 1998) to small shrubs and stem succulents (Lourteig, 1995). All species native to southern Africa are geophytes that bear true bulbs with diverse morphological attributes (Salter, 1944; A. K. Gebregziabher, unpublished manuscript). Additionally, all southern African members of *Oxalis* still exhibits tristylly, a breeding system characterised by a genetic polymorphism in which three floral morphs (long, mid and short, according to stigma height) are present within populations (Salter, 1944). It is described as an out-crossing mechanism that limits seed production to pollination between different morph types, which in turn reduces the negative effects of close inbreeding within populations (Barrett, 1992; Weller, 1992).

In South Africa, *Oxalis* is represented by ca. 210 species (ca. 270 taxa) (Dreyer & Makgakga, 2003). The majority of these species are concentrated in the southern and south-western parts of the Western Cape Province (Salter, 1944; Oberlander & al., 2002), an area included within the Cape Region (Cowling & Hilton-Taylor, 1997). From a conservation perspective, this region is of particular importance as it has been identified as one of the world's biodiversity hotspots based largely on its unique plant diversity (Myers & al., 2000). While some *Oxalis* species are very common, often with invasive tendencies, approximately 25% of the southern African *Oxalis* taxa are rare with highly localized distributions (Hilton-Taylor, 1996).

The question of why some species are rare whilst others are common and/or weedy has become an important research focus in conservation biology (Burne & al., 2003). In following Primack (1993) and Pullin (2002), a species may be considered rare if it displays one or more of the following characteristics: (i) if it occupies a narrow geographical distribution range, (ii) if it has highly specific habitat requirements, or (iii) if it is found only in small populations. Several *Oxalis* species are characterized by these criteria (Hilton-Taylor, 1996).

In the context of the endangered nature of many of these *Oxalis* taxa, it is critical to identify the spatial distribution of genetic variation across species and populations; information that will contribute to long-term survival of the group. Observed phylogeographic patterns are the result

of historic (and to a lesser extent current) space-time processes. These include current levels of gene flow amongst isolated populations, historic migrations and past demographic changes (see Avise, 1994, 2000 and references therein). These kinds of information are readily inferred from the relationships among intra- and interspecific DNA sequences. Additionally, genetic analyses can address whether populations represent units with independent evolutionary trajectories (or Evolutionary Significant Units; ESU) and provide insights into the evolutionary processes that determine the degree to which they are genetically distinct.

One of the most problematic questions in conservation biology when faced with shared haplotypes between presumed distinct lineages or species is to separate short divergence times with low gene flow from long divergence times with moderate gene flow. In the former instance, one could argue different evolutionary trajectories for taxa whereas this would be inappropriate when current gene flow is moderate to high. These difficulties of separating (short-term) isolation from recurrent gene flow are largely overcome through a combined maximum likelihood and Bayesian coalescent approach (Nielsen & Wakeley, 2001), where population parameters such as migration rate, effective population size as well as the time of population divergence are estimated simultaneously.

The aim of this paper is to assess the degree of genetic differentiation between two rare and highly localized species, namely *O. hygrophylla* Dreyer and *O. oligophylla* Salter, as well as the more widespread *O. tomentosa* L.f. We employed sequence data from the chloroplast intergenic spacer *trnH-psbA* to assess population parameters including the level of gene flow among these well-supported monophyletic *Oxalis* lineages (Oberlander & al., 2004). For comparative purposes, we also include the distantly related *O. purpurea* L., one of the most widely distributed species in South Africa.

MATERIAL AND METHODS

Study species. — *Oxalis tomentosa* is common and well-represented all over the Western Cape Province (South Africa), but is mostly restricted to shale-derived clay soils supporting renosterveld vegetation (J. Zietsman, personal observation). Although widely distributed, populations of this species are subjected to habitat fragmentation and disturbance within its natural distribution range, as large portions of renosterveld have been lost to agriculture (Kemper & al., 1998). *Oxalis oligophylla* is a Gifberg endemic (Salter, 1944) that grows in Mountain Fynbos. It occurs in rock crevices high on the Gifberg escarpment, where it is present

in extremely low densities. The Gifberg Mountain comprises mainly refugium Arid Fynbos surrounded by succulent karoo shrublands (J. Zietsman, personal observation). *Oxalis hygrophila* has only been recorded from moist, natural water seepage bands in the Pakhuis Pass (Salter, 1944; Kumwenda & al., 2004). *Oxalis purpurea* is a weedy species that is very abundant in the Cape Region and mostly grows in large, dense populations (Salter, 1944; Goldblatt & Manning, 2000). It has no specific habitat preferences and often colonizes very disturbed areas.

Samples. — Three populations of the widely distributed *O. tomentosa* (located at Darling, Saron and Elandsberg, Fig. 1) were included in this study. The Darling population is very disturbed and fragmented. An assessment of several reproductive traits (Chapter 2) suggests that clonal growth is the only mode for reproduction in this population. In contrast, *O. tomentosa* populations at the Saron and Elandsberg localities are large and relatively undisturbed. Seed production was observed within these populations, but they also display extensive levels of clonality, observed by the clumped distribution pattern of plants that form clusters of the same morph type (J. Zietsman, personal observation). Single populations of the rare *O. oligophylla* located in the Gifberg and *O. hygrophila* in the Pakhuis Pass (Fig. 1) were included. Both of these are natural populations, but *O. oligophylla* has an extremely low density. Due to the more solitary distribution of individuals, clonal growth is presumably low. The exact location of the type locality of *O. hygrophila* was visited on various occasions during October and November 2005, but not a single plant was found (J. Zietsman, personal observation). As a result, only one individual sampled during a previous visit was available for inclusion in this study. *Oxalis purpurea* is represented by a large, weedy population sampled in Stellenbosch (Fig. 1). Within this population, extensive levels of clonal growth accompany extensive seed production.

With the exception of *O. hygrophila*, for which only a single specimen was available, we included between 15 and 20 individual plants per population (Table 1). Since *Oxalis* is capable of vegetative reproduction via bulbil formation, collections were made at 1–2 meter intervals to prevent the sampling of clones. To further avoid the inclusion of vegetative samples, only a single individual was sampled per clump. Sampled material were dried and stored in silica gel matrix (Merck Laboratory Supplies (Pty) Ltd, Darmstadt, Germany).



Fig. 1. Geographical locations of the studied *Oxalis* populations in the Western Cape, South Africa.

Laboratory methodology. — DNA was extracted from 0.01–0.02 gram of dried leaf tissue using the 2X CTAB extraction protocol adapted from Doyle & Doyle (1987). In short, leaf tissue was ground in liquid nitrogen with a mortar and pestle and incubated in 2X CTAB isolation buffer at 60° C. Chloroform-isoamylalcohol (24:1 v/v) were added and centrifuged to separate a DNA containing aqueous phase. Cold isopropanol (–20° C) was used to precipitate nucleic acids and subsequently washed with a buffer containing 1 part of 40 mM ammonium acetate to 3 parts of ethanol by volume. The dried precipitants were resuspended in TE buffer.

To investigate the spatial distribution of genetic variation among populations, we employed the *trnH-psbA* chloroplast spacer marker. This region was amplified using the primers *trnH* (GUG) and *psbA* described by Hamilton (1999). PCR reactions were carried out in 30 µl reaction volumes and included 2 µl of genomic DNA, 3 µl of a 10X reaction buffer, 3 µl of 2 mM MgCl₂,

3 µl of 1 mM dNTP solution, 3 pmol of each primer (trnH (GUG) and psbA) and 1 unit of Taq polymerase (Super-Therm). The final volume was adjusted with deionised distilled water. The cycling parameters included an initial denaturation step at 96° C for 5 minutes followed by 30 cycles of 96° C for 30 seconds, 50° C for 30 seconds and 72° C for 40 seconds. A final extension step at 72° C for 5 minutes completed the reactions. PCR products were purified with the Wizard SV Gel and PCR clean-up system (Promega, U.S.A.) according to the manufacturer's recommendations.

Amplicons were cycle sequenced using BigDye chemistry (Applied Biosystems, U.S.A.). Unincorporated dye label was removed by sephadex columns before the samples were run on an ABI 3100 automated sequencer (Applied Biosystems, U.S.A.). Electropherograms of the raw data were manually checked and edited with Sequence Editor™ software (Applied Biosystems, U.S.A.). All *Oxalis* sequences generated in this study were deposited in GenBank (EF040587 – EF040597).

Sequence analyses. — Sequences were aligned with Clustal X (Thompson & al., 1997) using the multiple alignment mode and checked manually. Several insertions / deletions (indels) of various sizes were present in our data. We assumed that these indels represent single events rather than multiple events. In following Jansen van Vuuren & Robinson (1997), we coded all indels as single characters (see Results for detail discussion). PAUP* (Swofford, 2001) was used to assess the nucleotide composition and the number of parsimony-informative sites. Haplotype and nucleotide diversities were calculated in Arlequin 3 (Excoffier & al., 2005). Haplotypes were identified using MacClade 4 (Maddison & Maddison, 2000) and verified in Arlequin 3.

To depict the evolutionary relationship among species and populations, we followed a hierarchical approach. At the species level, phylogenetic trees were constructed for all taxa using both a parsimony and maximum likelihood approach. Parsimony analyses were based on heuristic searches with 100 random additions of taxa and TBR branch swapping. Gaps were considered as a 5th character state. The evolutionary model that best fitted our data (HKY; Hasegawa & al., 1985) was determined with Modeltest v3.7 (Posada & Crandall, 1998). To assess the robustness of resultant topologies, we obtained 1000 bootstrap replications for parsimony and maximum likelihood analyses. At the population level we constructed a

minimum spanning network to depict relationships among haplotypes using TCS (Clement & al., 2000).

To distinguish historical high levels of gene flow from recent population divergence, we followed a coalescence approach. MDIV (Nielson & Wakeley, 2001) was used to calculate theta ($\Theta = N_e\mu$), directional migration or gene flow ($M = N_e m$), divergence time ($T = t/N_e$) and the time to the most recent common ancestor (TMRCAs = $t\mu$) (N_e represents the effective population size, t represents the generation time, and μ represents the per locus mutation rate). The 95% credibility intervals were calculated whenever possible. To ensure convergence of the ergotic values, pairwise simulations were run for 2, 5, and 50 million generations with Mmax set at 5 or 10, while Tmax was set at 10 or 100. The analyses were conducted under the HKY (Hasegawa & al., 1985) finite sites model. Pairwise comparisons were confined to populations and species with shared haplotypes. For the calculations we assumed a generation time of one year. To our knowledge, very little is known about the mutation rate in the *trnH-psbA* chloroplast intergenic marker. Richardson and co-workers (2001) accepted a mutation rate of 0.0023% per million years (8.24×10^{-9} substitutions per site per year) for *Aichryson* (Crassulaceae) for the chloroplast *trnL-F* intergenic spacer. An investigation by Shaw & al. (2005) revealed that the *trnH-psbA* spacer was the second-most variable on a percent basis compared to 20 other non-coding chloroplast DNA regions. This investigation included the *trnL-F* intergenic spacer, which proved to be one of the least variable non-coding chloroplast regions. We assume that this region will therefore also evolve significantly slower than the *trnH-psbA* region employed in our study. In the absence of any other data, we assumed the mutation rate calculated for *trnL-F* as the lower boundary with an upper mutation rate boundary of 1% per million years (2.3×10^{-6} substitutions per site per year). However, given the uncertainty as to the exact mutation rate, our estimates should be taken as relative rather than absolute values. T , indicating the time of gene divergence, and TMRCAs, an indication of population divergence, were multiplied by their respective Θ values to correct for different effective population sizes.

RESULTS

Genetic diversity, indel treatment and haplotype frequencies. — Our final data set comprised 374 bp of the chloroplast *trnH-psbA* intergenic spacer for 91 specimens sampled from four species (Table 1 and Fig. 1). In total, six populations were sampled which included the rare and localized *O. oligophylla* ($n = 20$) and *O. hygrophila* ($n = 1$) as well as three geographically separated populations of *O. tomentosa* (Saron, $n = 20$; Elandsberg, $n = 15$;

Darling, n = 20). For comparative purposes we included the weedy *O. purpurea* (Stellenbosch, n = 15). Our data showed a nucleotide bias towards A (0.462) and T (0.324) with a noticeably lower C (0.115) and G (0.099) content. Homogeneity of base frequencies across taxa was not rejected ($p = 1$). Of the 374 bp, 346 characters were constant. The 28 variable characters were all parsimony informative. The transition: transversion ratio was estimated at 1:1.

Our data set (including the distantly related *O. purpurea*) contained a large number of indels varying in length between 1 bp and 112 bp. Although most of these were introduced to align *O. purpurea* with the other three species, indels also had to be introduced in the alignment of ingroup taxa including between the *O. tomentosa* populations. We argue that it is highly unlikely that an indel of, for instance 10 characters would represent 10 independent events, but rather reflect a single insertion / deletion event. In order to avoid any bias in our results, all gaps were recoded as single characters, reducing our data set to 230 bp. The coalescence methodology, implemented in MDIV, does not recognize indels as characters. Since we firmly believe that these indel characters add valuable information to our data (indeed, the majority of the information in the data are indel events), we recoded all indel characters as nucleotides in our coalescent analyses.

Table 1. Populations, number of specimens included per population, haplotypes distribution across populations as well as haplotype (gene) and nucleotide diversities is shown.

Species	Sampling locality	N	Haplotypes	h	π
<i>O. oligophylla</i>	Gifberg	20	A (n=15), B (n=5)	0.395 ± 0.101	0.022 ± 0.013
<i>O. hygrophila</i>	Pakhuis Pass	1	H (n=1)	1	0
<i>O. tomentosa</i>	Saron	20	B (n=16), D (n=3), I (n=1)	0.352 ± 0.123	0.011
	Elandsberg	15	B (n=15)	0	0
	Darling	20	C (n=20)	0	0
<i>O. purpurea</i>	Stellenbosch	15	E (n=6), F (n=3), G (n=4), J (n=1), K (n=1)	0.771 ± 0.072	0.011 ± 0.007

Eleven haplotypes were found for the 91 *Oxalis* specimens. The distribution of haplotypes across the different populations is indicated in Table 1. A shared haplotype (haplotype B) was found for the Saron and Elandsberg populations of *O. tomentosa*. Interestingly, and perhaps somewhat unexpectedly, this haplotype B is also shared between *O. oligophylla* and *O. tomentosa*. Five different haplotypes characterized the *O. purpurea* population from Stellenbosch. The high genetic diversity found in *O. purpurea* compared to the other species is reflected in their haplotype diversity values (see Table 1).

Phylogenetic relationships based on haplotypes. — To understand the evolutionary processes driving speciation in *Oxalis*, and to contribute knowledge towards determining why some species are localized and rare whilst others appear widespread and almost invasive, we reconstructed the phylogenetic relationships among the six populations included in this study. Irrespective of the method of analyses (parsimony or maximum likelihood), we obtained near identical results. For parsimony analyses, a single most parsimonious tree of 55 steps was found (CI = 0.865) (Fig. 2). It comprises three well-supported clades that correspond largely to *O. purpurea*, *O. tomentosa* and *O. oligophylla* / *O. hygrophila*. Sequence divergence values between clades I (haplotypes A, H, D) and II (B, I, C) was, on average, 1.8% compared to divergence values within clades which were always < 1%. Divergence values within *O. purpurea* were similarly low (< 1%); however, this weedy species was separated from the other clades by an average divergence of 10.8%. The maximum likelihood topology differed only in the placement of haplotype D within clade II rather than clade I. This difference in tree topologies is most likely the result of the way in which these different analyses methods treat gap characters.

At the population level, we constructed a minimum spanning network among haplotypes. The 95% connection limit was set at 6 steps. We could therefore not include all haplotypes into a single network. Rather, three networks resulted (Fig. 2) which corresponded to the three clades detected in our parsimony analyses.

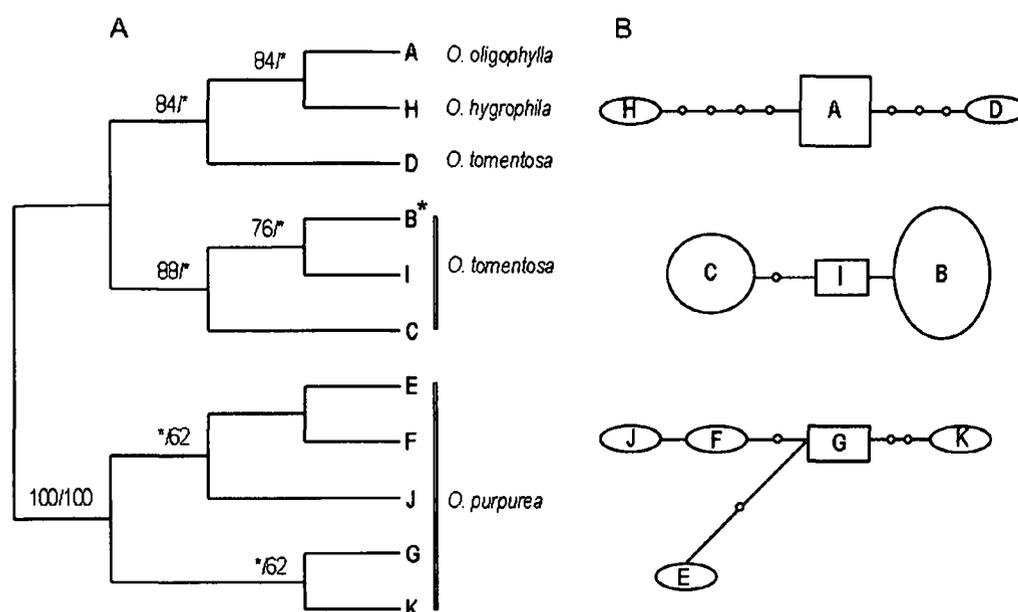


Fig. 2. (A) Parsimony analyses recovered a single most parsimonious tree of 55 steps (CI = 0.865). Bootstrap support (parsimony / maximum likelihood), following 1000 replicates, is indicated above branches. * denotes a bootstrap value below 60%. (B) The minimum spanning network, depicting the least number of mutational steps separating haplotypes, is shown. The 95% connection limit was set at 6 steps, resulting in three separate networks with congruence to the parsimony topology.

Coalescent modelling. — All analyses were run for 2, 5 and 50 million generations and in all cases estimates of theta, T and M were very similar. Migration rates among populations were invariably low (Table 2) with less than 1 migrant detected between *O. oligophylla*, *O. tomentosa* (Saron) and *O. tomentosa* (Elandsberg). θ , an indicator of population size, was relatively low and varied between 0.245 (*O. oligophylla* vs. *O. tomentosa* Elandsberg) and 1.072 (*O. oligophylla* vs. *O. tomentosa* Saron). In our calculations, we assumed a mutation rate of between 0.0023% and 1% per million years and a generation time of 1 year (the true mutation rate is likely to be closer to the upper boundary of 1% than the lower boundary). As expected, effective population sizes were relatively small (assuming a mutation rate of 1% per million years) and varied between 233 042 (for *O. oligophylla* and *O. tomentosa* Saron combined) and 53 260 (for *O. oligophylla* and *O. tomentosa* Elandsberg combined) (Table 2). When we tried to model the distribution of T, we encountered several problems in that the posterior distribution did not form a distinct peak with an asymptote that converged to zero. This would happen when the Monte Carlo variance for T is large combined with a relatively flat likelihood surface making reliable estimates impossible. Also and perhaps more importantly, the integrated likelihood value may have several peaks which can be explained when there were only a limited number of migration

events during the history of the species / populations (see Fig. 3c in Nielsen & Wakeley, 2001). It was therefore impossible for us to obtain any reasonable estimates of T and TMRCA.

Table 2. MDIV results estimated from *trnH/psbA* data for selected populations. The estimates of θ (and consequently N_e) and migration rates ($M = 2N_e m$) are indicated. We assumed a mutation rate of between 0.0023% (lower boundary) and 1% (upper boundary) per million years and a generation time of 1 year. The 95% credibility intervals were calculated whenever possible and are indicated between brackets. ** - values could not be estimated since the parameter did not converge back to zero.

Population 1	Population 2	θ	N_e (0.0023%)	N_e (1%)	M (Gene flow)
<i>O. oligophylla</i>	<i>O. tomentosa</i> (Saron)	1.072 (0.530/2.448)	101,323,251 (50,094,517/ 231,379,962)	233,043 (15,217/ 532,173)	0.200 (0.070/ 2.240)
<i>O. oligophylla</i>	<i>O. tomentosa</i> (Elandsberg)	0.245 (0.082/1.055)	23,156,899 (7,750,472/ 99,716,446)	53,260 (17,826/ 299,347)	0.08 (** /1.6)
<i>O. tomentosa</i> (Elandsberg)	<i>O. tomentosa</i> (Saron)	0.816 (0.373/ 1.993)	77,126,654 (35,255,198/ 188,374,291)	177,391 (81,086/ 433,260)	**

DISCUSSION

Phylogeographic patterns. — Our investigation of the spatial distribution of genetic variation in four *Oxalis* species revealed three well-supported clades (Fig. 2). These clades are, however, not strictly congruent with current taxonomy. This results from the clustering of haplotype D (identified in *O. tomentosa* sampled at Saron) with haplotypes A (*O. oligophylla*) and H (*O. hygrophila*), rather than with haplotypes B, I, and C (which characterize *O. tomentosa* populations). More importantly, our analyses further revealed the presence of shared haplotypes between *O. oligophylla* and *O. tomentosa*, where haplotype B characterizes specimens from the Gifberg (*O. oligophylla*), Saron (*O. tomentosa*) as well as Elandsberg (*O. tomentosa*).

Species that are not reciprocally monophyletic, in combination with a pattern of shared haplotypes, could indicate (high levels of) current gene flow between species. We strongly argue that this is not the case for *Oxalis*, and that the phylogeographic patterns evident in this genus are the result of shared ancestral polymorphism between species. Otherwise put, the observed pattern could be remnants of recent evolutionary divergence events with insufficient

time for populations / species to reach a state of reciprocal monophyly. Although we could not determine the time of population divergence (see Results for explanation), coalescent modelling unequivocally indicates very low levels of gene flow between species with the number of migrants exchanged between populations being invariably less than 1 migrant per generation (Table 2). Our result of limited gene flow is further supported by the geographical (spatial) separation (approximately 300 km separate populations) of these species together with short dispersal distance of seeds. *Oxalis* seeds are explosively dehisced from the capsule through rupture of the outer testa layer (Salter, 1944). This results in highly localized seed dispersal, with a dispersal distance of no more than 2 meters. Gene flow is further restricted by the complex tristylous breeding system, which limits successful pollination events even within a population (Ornduff, 1964) and could thus further complicate pollination events between two species. This is further corroborated by the absence of recorded occurrence of natural interspecific hybrids (Salter, 1944).

Low levels of genetic variation characterized both the *O. oligophylla* population as well as the three *O. tomentosa* populations. For example, a single haplotype characterized all specimens included from the Darling and Elandsberg populations of *O. tomentosa* (Table 1). Genetic variation within a particular population may be negatively influenced by restrictions to seed production, as equal availability of morph types as well as an effective pollen vector is required for the tristylous breeding system to function effectively (Ornduff, 1964). Failure of these requirements mostly leads to no natural seed production. This may indeed be the case for the *O. tomentosa* populations, and perhaps more specifically for the Darling population, where vegetative reproduction was observed to be the only mode of reproduction. These low levels of genetic variation stand in sharp contrast to the much higher levels of genetic variation observed in the weedy *O. purpurea*, where five haplotypes characterized 15 specimens. Importantly, the success of weedy species is often linked to higher levels of genetic variation (Karron, 1987).

Conservation implications. — From a conservation perspective, small populations are particular vulnerable to extinction due to low genetic variation, inbreeding depression and stochastic events, amongst others (Primack, 1993). Patterns or levels of genetic diversity may have a significant influence on the long-term persistence of local populations, and revealing such information is important in protecting rare species (Gustafsson & Sjögren-Gulve, 2002). Our estimates for theta and N_e should be taken only as estimates, since both are heavily influenced by the mutation rate (which in itself is a gestimate; see Results section). Similarly, N_e

is influenced by extensive vegetative reproduction via clonal growth, given that flowering is strongly influenced by temperature and rainfall (Dreyer & al., 2006). Furthermore sexual reproduction in *Oxalis* is also influenced by the complex tristylous breeding system, as seed production is limited to highly specific pollination events between specific morph types (Ornduff, 1964; Barrett, 1992; Weller, 1992). Theoretically, our estimates of the effective population sizes is small and given the above mentioned implications of *Oxalis* reproductive biology, the number of reproducing individuals is not equal to reproductive success. Although the rare species (*O. oligophylla* and/or *O. hygrophila*) are not monophyletic in our study, they appear to be recent endemics that are naturally rare and should be conserved as recently diverged species with different evolutionary trajectories.

A combination of various biological attributes (lack of specific habitat requirements, efficient ability to reproduce both vegetatively and through high levels of seed production, an extended flowering period, geographical flower colour variation etc.) may explain the wide distribution and abundance of *O. purpurea*. The high level of genetic variability found in this species relative to the genetic variability of the more restricted species was therefore not completely unexpected. This high level of genetic variability, as detected from the 15 individuals studied, may reflect the potential of this species to colonize such a variety of habitats. In contrast to *O. purpurea*, the low levels of genetic variability in other species might compel it to a narrow geographical distribution range and specific habitat patches as it is unable to adapt to different environmental conditions. These low levels of genetic variability detected in the studied species appear to result from a combination of small population sizes, restrictions to sexual reproduction and also a limited availability of their species specific habitats.

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CHAPTER 4

CONCLUSIONS

The study of rarity is critical for the conservation of rare plants and essential for the long-term preservation of biodiversity. The key to protecting and managing a rare or endangered species is to have a sound understanding of its biological relationship with its environment. To this end we need to locate and identify all extant populations of such species, and determine the current condition of and potential threats to its natural habitat throughout the range of the species.

Rarity appears to be a unique response to the unique environmental conditions of each of the species investigated in this study. Results revealed that the reproductive success of certain *Oxalis* species is limited by the complex tristylous breeding. Species confined to small isolated populations may lack an effective pollen vector and equal availability of three different morph types within populations, which is essential for seed production in tristylous populations. Some species overcome these limitations through relaxation of the expression of tristily, suggesting that reproductive assurance may be more important to such species than the genetic variability attained through cross-pollination. Other rare/endangered *Oxalis* species appear not to be negatively affected by tristily expression, such that it still appears favorable to retain all aspects of the tristylous breeding system.

O. giftbergensis and *O. oreophila* are not restricted by the tristylous breeding system and do not appear to be under immediate threat, provided that their habitat can be preserved. *O. amblyosepala* is potentially under threat due to its confinement to small isolated populations, consequent limitations to sexual reproduction imposed by the tristylous breeding system and the very distinct and fragile habitat preferred by this species. Selected populations of *O. natans* could be under threat as a result of disturbance, competition for pollinators and the limitations to sexual reproduction resulting from fragmentation, habitat loss and possibly a low fecundity as a result of inbreeding depression. Although these species are able to reproduce vegetatively, limitations to sexual reproduction can negatively impact on the long term persistence of such species, as population persistence is normally still dependent on seedling development. *O. natans* do not appear to be

limited by its breeding system under natural conditions (studied population *O. natans* 2). The rare/endangered *O. comptonii* and *O. oculifera* are able to escape some of the limitations associated with small population sizes through tristylous breakdown, while still retaining the potential to out-cross. As selfing is only beneficial over the short-term (negative effects of inbreeding), the retained ability to out-cross can be particularly advantageous for species such as *O. comptonii* and *O. oculifera* in which population size and density were observed to be extremely variable according to variable rainfall patterns. Selfing may thus counter insufficient cross-pollination in unsuitable years, while the potential to out-cross when conditions are more favorable, will counter low genetic variability imposed by selfing.

The future survival of *O. hygrophila* is currently immensely threatened, since no plants could be found in the only known locality of this species. This locality needs to be monitored, especially when rainfall patterns similar to 2001 (during which flowering was recorded in November) (Chapter 2) are observed to analyse the status of this population and to implement conservation strategies for this species. Conservation efforts should also attempt to overcome limitations to reproductive success and should try to increase out-crossing opportunities, especially in populations that show reduced seed production in the wild (*O. amblyosepala*, *O. natans* 1 and *O. tomentosa* 1). A possible method would be artificial hand-pollinations between different floral morphs in the field (attempt to mimic natural cross-pollination). This can also be used to assess whether a population still has the potential to produce seeds in the wild. Artificial cross-pollinations may also be used to increase gene flow among isolated populations with presumably low genetic variability. Another possibility would be to cultivate plants of the different morph types under controlled greenhouse conditions and then to collect seeds to be used in reseeded trials for re-establishment in the wild.

As most of the study species are limited by their highly specific habitat requirements, it is of immediate importance to conserve these habitats. Most threatened are those species confined to the fragile seasonally waterlogged habitats, as climate appears to play an important role in the abundance of such species, keeping predictions of climate change (Chapter 2 Discussion; Ecology) in mind. The identification of key characteristics of the habitats of rare/endangered species will provide the ability to predict additional sites in which populations of the selected species may be found. This may aid location and conservation of more populations and aid future conservation efforts as well as the prioritization of conservation sites.

Small populations are vulnerable to extinction for a number of reasons, including especially low genetic variability (Chapter 1). Assessments of the patterns and levels of genetic variation are thus critically important aspects to consider when attempting to protect and conserve a rare species. Both *O. oligophylla* and *O. tomentosa* are characterized by low levels of genetic variation (Chapter 3). In this case these low levels of genetic variability in the naturally rare species (*O. oligophylla*) may be the result of its highly specific habitat requirements, which confine it to small, isolated populations (limited available habitat). These conditions will prevent such a species from adapting to changing environmental conditions or to colonize larger ranges. Restrictions to sexual reproduction in small populations and extensive clonal propagation can further lower levels of genetic variation. This was evident in *O. tomentosa* (1) (Chapter 3) for which clonal growth appears to be the only way to persist. Results obtained for *O. tomentosa* revealed that specific populations can be in danger regardless of the wide distribution range of the species as a whole (Chapter 2). Many other populations of this species could face similar threats and it is therefore important to conserve populations of widespread species in fragmented landscapes. For the widespread *O. tomentosa*, the noticeably low levels of genetic variability (Chapter 3) may reflect the effects of Renosterveld fragmentation with consequent reduction in population sizes and increased isolation.

As rarity appears to be the result of a combination of different factors for most of the studied species (small population sizes, low genetic variability, restrictions by a complex breeding system, distinct and fragile habitats, etc.), a combination of various biological attributes may explain the wide distribution of others. The weedy species *O. purpurea* displays extremely high levels of genetic variability compared to the rare species (Chapter 3). This high genetic variability may enable this species to adapt to different environmental conditions. The lack of specific habitat requirements, variation in flower colour, an extended flowering period, efficient seed production, the ability to vary levels of tristyly expression and extensive clonal propagation, which may also facilitate the colonization of new habitats, all appear to contribute to the wide distribution of *O. purpurea*.

The present study confers potential threats and limitations to *Oxalis* populations and imposes future research in the managements of rare *Oxalis* species. Intensive demographic studies are essential to the managements of rare species. This can be used in future research to identify limiting factors for

a specific threatened species and also to determine whether populations are declining. The incorporation of population genetic studies that include levels of genetic variability and gene flow among population will also aid predictions on the long-term survival prospects of rare species. Comparisons of multiple attributes of rare species with each other and with closely related but more abundant relatives do provide insights on the processes and potential causes underlying the dynamics of rare plants.