

A study of tristylly in South African *Oxalis*

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own, original work, that I am the owner of the copyright thereof and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

This research is a compilation of studies that assessed essential elements of the expression of the tristylous system in members of the genus *Oxalis* in South Africa. The research is unique in that it is the first comprehensive study on tristily expression conducted at a generic level. It provides extensive ground work for understanding the tristylous system, paving the way for comparison with other families, genera and species that also express tristily.

The first aim was to assess tristily through a study of floral morphs at population level. For this, 104 populations were examined across the Western and Northern Cape Provinces of South Africa, including 58 different *Oxalis* species. *Oxalis* in South Africa presented considerable variation in the degree of floral morph expression. Stochastic events are likely to explain this variation, but no distinct pattern emerged when relating the data to explanatory variables collected.

The second aim was to assess the main characters that influence pollen flow in the tristylous system. The morphology of sexual reproductive organs was evaluated, where all organ level heights were measured including all morphs present in a population. Secondly, pollen sizes were measured, which are known to differ between anthers of tristylous species. In most morphs of *Oxalis* flowers analyzed three levels of sexual reproductive organs were found, with evident separation and reciprocity between them. However, in some morphs there was a lack of reciprocity and/or separation of reproductive organ levels and this could not be related to the morph equilibrium in the populations where it was found.

In a parallel analysis patterns of tristylous expression among South African *Oxalis* species were compared to patterns of tristylous breakdown among American members of the genus. The South African species *O. glabra* Thumb, *O. nidulans* E. & Z., *O. pes-caprae* L. and *O. purpurea* L. were compared to the American species *O. alpina* (Rose) Knuth. It evaluated and compared the scarcest morph, arrangement of sexual reproductive organs (organ height ratios), geographic location, environmental disturbance and distribution of each species. South African *Oxalis* species did not show directionality in terms of the morph lost, but indeed lacked a variety of different morphs in different populations. No clear relationship between geographic location and anisopleth could be detected.

The next chapter represents a specific case study of the reproductive organ arrangement in members of *Oxalis* section *Sagittatae*. Detailed analyses of their morphology, incompatibility reactions and morph equilibrium were undertaken. This system, only observed until present in section *Sagittatae* in South African *Oxalis* members, is here termed as three-dimensional tristylous. This system relies on successful cross-fertilization through reproductive organ arrangement, quite independent from the presence or absence of self-incompatibility.

The final chapter analyses the impact of tristylous and polyploidy on the breeding system of *Oxalis hirta* L. var. *tubiflora* (Jacq.) Salter. Results from a complete hand-pollination experiment revealed that the expression of self-incompatibility in *Oxalis hirta* var. *tubiflora* compares to a typical tristylous species. Polyploidy does not interfere with the expression of tristylous or the fertility of *Oxalis hirta* var. *tubiflora*.

OPSOMMING

Hierdie navorsing verteenwoordig 'n samestelling van studies wat die belangrikste komponente van die uitdrukking van die tristylie sisteem in Suid-Afrikaanse lede van die genus *Oxalis* ondersoek het. Die navorsing is uniek daarin dat dit die eerste omvattende ondersoek van tristylie-uitdrukking verteenwoordig wat op 'n generiese vlak onderneem is. Dit verskaf 'n stewige basis vir die begrip van die tristylie sisteem, en baan die weg vir vergelyking met ander families, genera en spesies wat ook tristylie uitdruk.

Die eerste mikpunt was om tristylie te ondersoek aan die hand van 'n studie van blom-morf verteenteenwoordiging op die populasie-vlak. Hiervoor is 104 populasies, verteenteenwoordigend van 58 *Oxalis* spesies, regoor die Weskaap en Noordkaap Provinsies ondersoek. Suid-Afrikaanse *Oxalis* openbaar geweldige variasie in die graad van blom-morf uitdrukking. Stochastiese gebeure verduidelik waarskynlik hierdie variasie die beste, maar geen duidelike patroon het uitgestaan toe hierdie data vergelyk is met ander potensieel verduidelikende veranderlikes wat versamel is nie.

Die tweede mikpunt was om die hoofkenmerke wat stuifmeel-verspreiding in die tristylie sisteem beïnvloed te ondersoek. Die morfologie van geslagtelike voortplantingstrukture is ondersoek, waartydens alle orgaan hoogtes gemeet is van alle morf-tipes binne elke populasie. Stuifmeelkorrel-groottes is tweedens gemeet, aangesien dit bekend is dat dit varieer tussen verskillende helmknoppe van spesies met tristylie. In die meeste *Oxalis* blom-morfe wat ondersoek is, is drie vlakke van geslagstrukture gevind, met duidelike skeiding en repositieit tussen hulle. Enkele blom-morfe het egter ontbreek aan repositieit en/of skeiding van

geslagsorgaan vlakke, en dit kon nie gekorreleer word met blom-morf ekwilibria in die populasies waarin dit voorgekom het nie.

In 'n parallele analise is patrone van tristylie-uitdrukking tussen Suid-Afrikaanse *Oxalis* spesies vergelyk met patrone van tristylie-afbraak tussen Amerikaanse lede van hierdie genus. Die Suid-Afrikaanse spesies *O. glabra* Thumb, *O. nidulans* E. & Z., *O. pes-caprae* L. en *O. purpurea* L. is vergelyk met die Amerikaanse spesie *O. alpina* (Rose) Knuth. Dit het die skaarste morf-tipe, die rangskikking van geslagsorgane (orgaan-hoogte verhoudings), geografiese lokasie, omgewingsversteurings en verspreidingspatrone van elke spesie ge-evalueer en vergelyk. Suid-Afrikaanse *Oxalis* spesies het geen direksionaliteit in terme van morf-verlies getoon nie, maar het wel ontbreek aan 'n verkeidenheid verskillende morfe in verskillende populasies. Geen duidelike verhouding tussen geografiese posisie en anisopleetiese balanse kon gevind word nie.

Die volgende artikel (hoofstuk) verteenwoordig 'n spesifieke gevalle-studie van geslagsorgaan-rangskikking in lede van *Oxalis* seksie *Sagittatae*. Gedetailleerde analises van hulle morfologie, self-onverenigbaarheidsreaksies en morf-ewewigte is onderneem. Hierdie sisteem, wat tot op die hede slegs in lede van seksie *Sagittatae* waargeneem is tussen Suid-Afrikaanse lede van *Oxalis*, is word hier beskryf as drie-dimensionele tristylie. Hierdie sisteem maak staat op suksesvolle kruis-bestuwing deur geslagsorgaan-rangskikking, heel onafhanklik van die aan- of afwesigheid van self-onverenigbaarheid.

Die finale artikel (hoofstuk) analiseer die impak van tristylie en poliploidie op die kruisingsstelsel van *Oxalis hirta* L. var. *tubiflora* (Jacq.) Salter. Resultate van 'n volledige handbestuivings-eksperiment in *Oxalis hirta* var. *tubiflora* vergelyk goed met 'n tipiese tristylie-uitdrukkende spesie. Poliploidie affekteer nie die uitdrukking van tristylie of die vrugbaarheid van *Oxalis hirta* var. *tubiflora* nie.

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1. Dissertation Structure

2. Tristyly in flowering plants:
A review

3. Morph representation in
Oxalis populations across the
Cape Floristic Region

4. Tristyly and sexual organ
morphology in South African
Oxalis

5. Patterns related to morph
frequency in tristylous *Oxalis*
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6. Three-dimensional
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7. Tristyly and self-
incompatibility in a polyploid
South African *Oxalis* species

8. Tristyly in the genus *Oxalis*
in South Africa: A review

CHAPTER 1 - DISSERTATION STRUCTURE

The experimental component of this research is presented in five self-contained scientific articles (chapters 3 to 7), each with their own aims, methods, and conclusions. Many of these chapters have been submitted for publication in national and international journals and are co-authored. I have indicated the names of co-authors in the overview below; therefore reference to “we” in these chapters refers to me as main author and instigator of the research, and to my co-authors. All chapters contribute to the overall understanding of the main objective, which is to gain a better understanding of the tristylous system in *Oxalis* species in South Africa.

Chapter 1 – Overview: The current chapter provides an overview of the dissertation structure, and includes a breakdown of roles and responsibilities for each chapter.

Chapter 2 - General introduction: This chapter comprises of a literature review that explores the current understanding of tristily and its function, including its occurrence in other families and genera, as well as a review of the main lines of research related to sexual organ polymorphisms. *This chapter was entirely the work of S.S. Siqueira. The refinement of this document was achieved by comments and suggestions made by my promoters L.L. Dreyer and K.J. Esler.*

Chapter 3 - This chapter explores the equilibrium of morphs in populations as a measure of tristily expression. More than one population of the same species were studied in some cases to identify possible intra-specific variations in tristily expression. *This chapter was designed by*

S.S. Siqueira with assistance from L.L. Dreyer and K.J. Esler. This chapter has been submitted to the journal Annals of the Missouri Botanical Gardens, and is currently under review.

Chapter 4 – This chapter explores the degree of sexual organ reciprocity through an analysis of their individual heights. These measurements were then compared to population morph frequency data obtained in Chapter 3 to explore possible associations between these two variables. In addition, pollen grain size was evaluated and compared to these two variables. *This chapter was designed by S.S. Siqueira with assistance from L.L. Dreyer and K.J. Esler. The verification and assistance with the interpretation of statistical analysis in this chapter was provided by F. Roets.*

Chapter 5 – This chapter compares patterns of tristily expression in four South African *Oxalis* species to breakdown patterns observed in *Oxalis alpina*, an American member of the genus. The distribution of each species was mapped and populations were explored. *S.S. Siqueira designed the framework of this paper, with input from L.L. Dreyer and K.J. Esler. It has been prepared for submission to the South African Journal of Botany and is currently under review.*

Chapter 6 – This chapter assesses the state of tristily expression in members of *Oxalis* section *Sagittatae* with focus on the three-dimensional arrangement of reproductive structures. This arrangement is reminiscent of a system described in *Linnum suffruticosum* L. The aim was to identify and illustrate the nature of the three-dimensional arrangement in this section and to experimentally test the level of self-incompatibility expressed in selected populations. *This chapter was designed by S.S. Siqueira with assistance from L.L. Dreyer and K.J. Esler.*

Chapter 7 – In this chapter the reproductive system of *O. hirta* var. *tubiflora*, a polyploid tristylous taxon with a suite of unusual biological attributes, is explored. Artificial pollination

experiments were performed in three populations of this variety and results were related to biological attributes of this taxon. *This chapter was designed by S.S. Siqueira with assistance from L.L. Dreyer and K.J. Esler.*

Chapter 8 –The overall findings of this research are summarized and discussed in this final chapter and it highlights essential characteristics that set tristylous expression among South African members of *Oxalis* apart from other heterostylous families and genera.

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4. Tristyly and sexual organ morphology in South African *Oxalis*

5. Patterns related to morph frequency in tristylous *Oxalis* species

6. Three-dimensional reciprocity in *Oxalis*

7. Tristyly and self-incompatibility in a polyploid South African *Oxalis* species

8. Tristyly in the genus *Oxalis* in South Africa: A review

Introduction

This chapter provides the key terminology relating to the subject of this dissertation, while summarising the current state of knowledge of tristily, including its occurrence in other families and genera. It also introduces the current main lines of research related to sexual organ polymorphisms.

Heterostyly

Heterostyly is the occurrence of flowers with different sexual organ arrangements in different plants of the same species (Darwin, 1877). It is a sexual polymorphism in which populations are composed of two (distyly) or three (tristyly) floral morphs with reciprocal arrangements of anthers and stigmas (reciprocal herkogamy) (Ganders, 1979).

Distyly presents two distinct floral morphs, the Long morph (Pin) and the Short morph (Thrum) (Dulberger, 1992) (Fig 2.1A). Anthers and stigmas of distylous plants are presented at equivalent heights in two alternate positions. In tristily, sexual organ arrangements result in three distinct floral morphs, with one set of stigmas and two sets of anthers alternating at three levels at equivalent heights (Barrett, 1992) (Fig 2.1B). The Long morph presents stigmas in the highest position, and anthers at the mid and short levels. The Short morph has its stigmas in the lowest position and the two sets of anthers above them, while the Mid morph has stigmas at the mid level between the two sets of anthers.

Heterostyly is distinct among other types of sexual polymorphisms. Stigma-height dimorphism and enantiostyly include two distinct floral morphs, but the main character differentiating

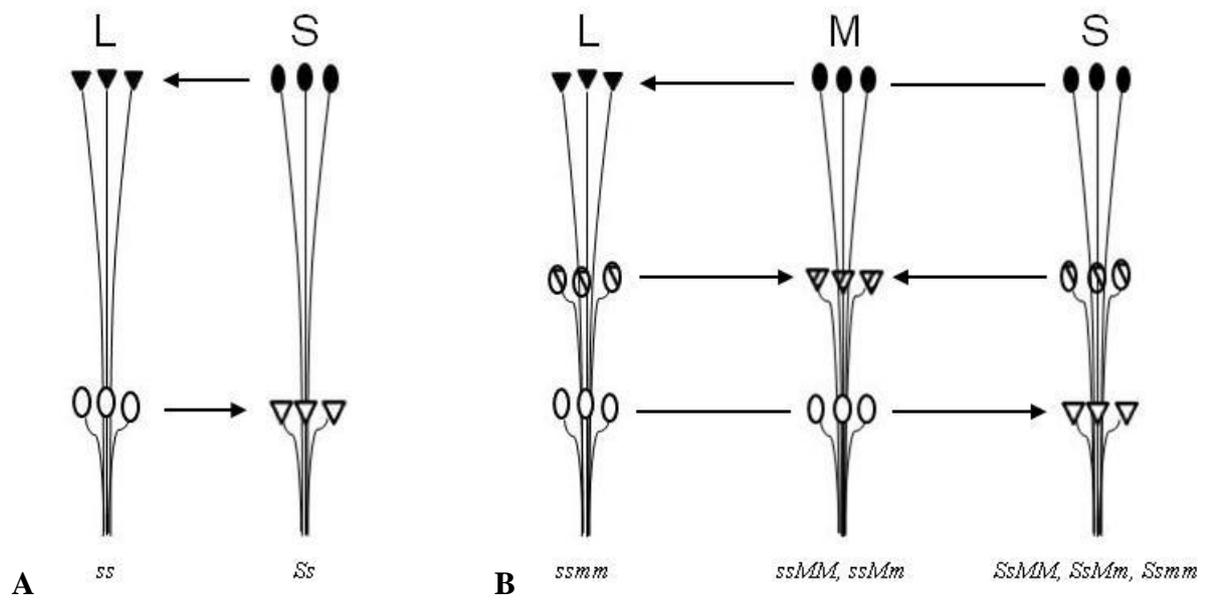


Fig. 2.1: The heterostylous polymorphisms: **A** distyly and **B** tristylly. L, M and S represent the long-, mid- and short-morphs, respectively. Upside-down triangles represent styles, while oval shapes represent anthers. Horizontal arrows indicate legitimate cross-pollinations. Genotypes for the floral morphs with the most common patterns of inheritance are indicated (Barrett and Shore, 2008)

these from distylous species is the lack of reciprocity of sexual organs (Barrett *et al.*, 2000). In stigma-height dimorphism the level of the anthers is maintained in both floral morphs, while only stigmas alternate in height. The two morphs are the Long, where stigmas are above the anthers, and the Short, where the stigmas are below them. In enantiostyly, the style is deflected away from the main axis of the flower either to the left or to the right, resulting in mirror image flowers.

Two other sexual polymorphisms have been described fairly recently, inversostyly and flexistyly and a variation of distyly (three-dimensional reciprocity); all presenting reciprocity of sexual organs in a different manner than that of heterostylous plants. Inversostyly was first described in *Hemimeris racemosa* (Houtt.) Merrill (Scrophulariaceae), in which zygomorphic flowers present a reciprocal arrangement of anthers and stigmas on a vertical plane, with no alteration in sexual organ heights (Pauw, 2005). The two morphs are named according to the stigma position based on the morphology of the petals, thus in the style-down morph, the style is positioned away from the oil-secreting pouch, and the two sets of anthers are positioned close to the pouch. This order is reversed in the style-up morph. Populations of this species comprise exclusively of these two morphs (inversostylous populations) or comprise exclusively of a third morph type in which stigmas and anthers are all clustered together in a down position of a disc-shaped flower (homostylous populations). Flexistyly was first described in the genus *Alpinia* (Zingiberaceae) (Li, 2001a, 2001b). In this case the stigma orientation alternates reciprocally in a zygomorphic flower by curving either upwards, away from the anthers (protandrous morph) or downwards towards the anthers (protogynous morph). Plants always express either one of these two morphs. Interestingly, the morphs reverse their arrangement according to the time of the day. A flower that is protandrous in the morning becomes protogynous in the afternoon, and the reverse happens in the protogynous flower. In this way, they alternate between male and female function as a result of style movement. Three-dimensional reciprocity was first described in

Linum suffruticosum L. (Linaceae) (Armbruster *et al.*, 2006). This plant presents a twisted distyly type of polymorphism, where styles and stamens bend during the development of the flower, achieving a three-dimensional arrangement. The two morphs, Long and Short, also have a reciprocal arrangement in the height of their organs as in distyly.

The key function of heterostyly is believed to be the promotion of cross-fertilization (Barrett, 2002). Reciprocal sex-organ positions in the morphs function to increase male fertility by actively promoting more precise pollen dispersal among plants than would occur in populations with uniform sexual organs and herkogamy. This is achieved by limiting functional interference between sexual organs, so reducing levels of male gamete wastage through self-pollination. The floral design reduces the possibilities of inbreeding depression by assisting the plants to exchange genetic material and maximizing parental fitness. Darwin (1877) described heterostyly as a system that facilitates insect mediated pollination and reduces pollen wastage. Most modern workers agree with Darwin on the interpretation of heterostyly as an outbreeding mechanism (for a review see Barrett, 1992). Armbruster *et al.* (2006) affirm three-dimensional heterostyly to be far more efficient in dispersing pollen because of the precision of placement of pollen on the insect's body.

Assessment of the morphology of a flower is imperative to understand the pollination process (Dulberger, 1992). For plants that rely on a vector to carry pollen, the arrangement of the sexual reproductive organs combined with the structure of the corolla, set the fate for success or failure of fertilization and therefore the chance of genetic recombination. In species where vectors are insects, there must be an adequate dimensional relationship between a flower and the insect for the pollination process to be successful. It has been proven that floral polymorphisms influence pollen flow in natural populations (Hernandez & Ornelas, 2007; Hodgins & Barrett, 2008).

Ganders (1979) pointed out that pollen flow is asymmetric and observed that small differences in

the positioning of stigmas and anthers can significantly change the rate of disassortative pollination. The arrangement of sexual organs combined with pollinator behaviour determines where pollen is deposited on the insect's body, supporting Darwin's (1877) interpretation on outbreeding (Wolfe & Barrett, 1987; Lloyd & Webb, 1992b).

Polymorphisms are reported in numerous families, with heterostyly alone reported in 28 angiosperm families, including both monocotyledonous and dicotyledonous families (Barrett, 2002). They can be restricted to individual species (e.g. in the Lamiaceae and Polemoniaceae; Barrett, 2002) or predominate at genus level (tristyly in *Oxalis*; Salter, 1944). Some families have different types of polymorphisms in different genera (Pontederiaceae - Barrett *et al.*, 2000), while others present different polymorphisms at species level (Amaryllidaceae, *Narcissus* - Graham & Barrett, 2004). For example, three-dimensional heterostyly is one of five polymorphisms known to occur in the Linaceae, while the discovery of inversostyly was the first report of a polymorphism expressed in the Scrophulariaceae (Armbruster *et al.*, 2006; Barrett *et al.*, 2000; Pauw, 2005).

Heterostyly is usually associated with other characteristics such as polymorphisms of stigma and pollen between the different organ levels (ancillary characters) and very often with a self-incompatibility system (Barrett, 1992). In early definitions of heterostyly, the arrangement of organs, the ancillary characters and the self-incompatibility system were all taken as parts of one system, which worked in combination as a syndrome (Lloyd & Webb, 1992a). Recent studies have, however, treated the components of the syndrome separately, associating the reciprocity of organs as the main character to define heterostyly (Lloyd & Webb, 1992a; Pérez-Barrales *et al.*, 2006).

The inheritance of heterostyly is regulated by diallelic loci that appear to control the entire syndrome of physiological and morphological traits (Barrett & Shore, 2008). In distyly there is a segregation of a dominant (*S*) and a recessive (*s*) allele governing the Long morph (*ss*) and Short morph (*Ss*). Tristyly inheritance involves two diallelic loci (*S* and *M*), with the *S*-locus epistatic to the *M*-locus (Lewis & Jones, 1992). Plants carrying the *S*-allele are of the Short morph, and those that are homozygous recessive at the *S*-locus are either of the Mid morph (*ssMM* and *ssMm*) or of the Long morph (*ssmm*). The loci are linked in most *Oxalis* species (Barrett & Shore, 2008). Additional loci and recessive epistasis occur as exceptions in some *Oxalis* species (Ganders, 1979; Trognitz & Hermann, 2001). The genetic system is expected to be maintained in a population by the equilibrium of morph types composing a polymorphism, however, several other factors influence successful reproduction, including pollen size and incompatibility reactions.

Tristyly

Tristyly is perhaps the most complex breeding system in flowering plants (Weller, 1992). It is unique in that two incompatibility reactions are found within a single individual. For each floral morph, pollen grains produced by each one of the two sets will only germinate and lead to seed production in a second floral morph, resulting in the complexity in number of compatible crosses. Due to reciprocal herkogamy, intermorph pollinations are favoured over intramorph transfers, because of segregated pollen deposition on different pollinator parts (Wolf & Barrett, 1989). Due to self-incompatibility, usually present in heterostylous species, only six of the 18 possible pollen transfers should result in fully successful fertilization.

Darwin (1877) classified a typical trimorphic species as one that manifests all aspects of the syndrome: three morph types in a population, present in equal ratios, accompanied by differences in the morphology of the pollen and stigma, and a functional self-incompatibility system.

Functional self-incompatibility restricts successful pollination to the transfer of pollen from anthers to stigmas of the same heights (legitimate pollinations), with intra-morph pollinations unable to produce a full set of seeds. Significant divergence from these expected characteristics are seen as breakdown of the syndrome and may pose hindrances to optimum exchange of genetic material through cross-pollination.

Isoplethic equilibrium, defined by Finney (1952) as equal representation of style length morphs, is the most accessible and easy way to rate the morphological expression of tristylous. It has been widely used in studies where the equilibrium was related to other parts of the syndrome, with comparisons been made across genera and families (Weller, 1992; Barrett *et al.*, 1997; Barrett *et al.*, 2000; Armbruster *et al.*, 2006). In tristylous species with heteromorphic incompatibility, a 1:1:1 morph ratio is expected in large populations at equilibrium because of frequency-dependent mating (Barrett *et al.*, 1997).

Tristylous has been recorded in the Amaryllidaceae, Connaraceae, Linaceae, Lythraceae, Oxalidaceae, Pontederiaceae and Thymelaceae (Barrett, 1993). In Lythraceae, tristylous is restricted to a few species in three of the 22 included genera, *Lythrum*, *Nesaea* and *Decodon* (Weller, 1992). Although most species in *Lythrum* section *Mesolythrum* have tristylous populations at equilibrium, section *Euhyssopifolia* includes both tristylous and distylous species. In this case distylous populations resulted from morph loss of original tristylous populations (Ornduff, 1979). The tristylous species *Lythrum junceum* Banks & Sol. displays differences in morph fertility and *L. salicaria* L. deviates from normal tristylous incompatibility reactions (Ganders, 1979). Several other species present losses of entire morphs, and this was related to different causes (*L. californicum* Torr. & Gray section *Euhyssopifolia* - short, *L. rotundifolia* L. section *Hochstetteria* - mid, *Nesaea* section *Heimiastrum* and *Pemphis acidula* J. R. & G. - Mid) (Weller, 1992). The tristylous species *Decodon verticillatus* (L.) Ell. lacks self-incompatibility

expression and also displays both trimorphic and dimorphic populations (Eckert & Mavraganis, 1996). In addition, it presents extensive clonality in the northern limit of its range (Eckert & Barrett, 1993).

Pontederiaceae is a small family including only 34 species in nine genera, of which only *Pontederia* and *Eichhornia* present tristylly (Weller, 1992). Some species in these genera display both loss of morphs and self-compatibility. *P. cordata* L., *P. sagittata* Presl and *P. rotundifolia* L. all lack morphs, which has been related to the same causes, but display varying degrees of self-incompatibility (Barrett, 1977b, Glover & Barrett, 1983; Weller, 1992). In all of them, self-incompatibility is stronger in the Short and Long morphs. *P. sagittata* shows under-representation of the Long morph in most of its populations. *P. cordata* lacks the Long morph and is over-represented by Short morphs. Tristylly is found in three species of *Eichhornia*, while the remaining five species are monomorphic (Weller, 1992). Populations of *Eichhornia azurea* Kunth include all three morphs, but not at isoplethic equilibrium. Long morphs have a high degree of self-incompatibility when pollen comes from the short anthers, but self-crosses with pollen from the mid-level anthers result in abundant seed production. In addition, *E. azurea* also has monomorphic populations, and presents semi-homostylous morphs (morphs with one set of anthers and stigmas at the same level, Lewis, 1954 - Fig 2.2). *E. crassipes* (Mart.) Solms. is similar, including trimorphic populations that are always anisoplethic. This species is very weedy and colonizes vast areas. Dimorphic and monomorphic populations are also found mainly in areas outside of the species' native range (Barrett, 1977a). Such populations consist of Long and Mid morphs, or Mid morphs only. It displays no self-incompatibility, and monomorphic populations produce seeds. *E. paniculata* (Spreng.) Solms-Laubach also has three morphs, with dimorphic and monomorphic expression occurring at the geographical margins. In addition, there are also monomorphic populations formed by semi-homostylous Mids, with a high degree of autogamy (Weller, 1992).

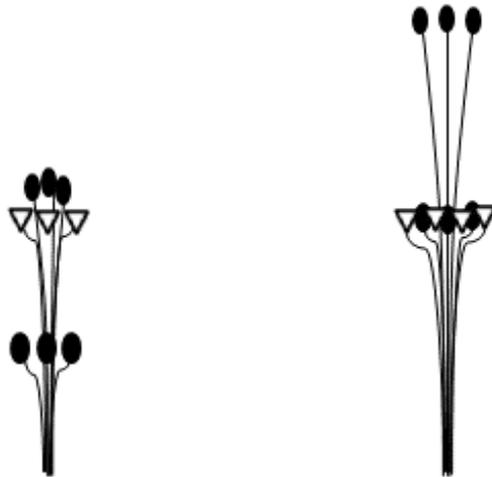


Fig. 2.2: Examples of semi-homostylous morphs. Upside-down triangles represent styles, while oval shapes represent anthers. Drawings were based on illustrations from Ornduff (1972).

In the Connaraceae tristily has been reported in *Agalea*, *Jollydora* and *Manotes* and trends in breeding systems in the family were similar to those documented in the Lythraceae and Oxalidaceae (Lemmens, 1989; Barrett, 1993).

Tristyly in Amaryllidaceae is expressed only in the species *Narcissus triandrus* L., where the majority of populations have all three morphs represented (Barrett *et al.*, 1997). *N. triandrus* differ among the tristylous species with no evidence of the typical heteromorphic self-incompatibility system and slight differences in anther and stigma positions in the Long morph. In addition, its populations have an excess of Long morphs, and display a gradual loss of Mid morphs that eventually culminate in distylous populations (Barrett *et al.*, 1997).

In Linaceae, tristily is only expressed in the extremely rare and endangered species *Hugonia serrata* Lam. native to the Mascarene Island and Mauritius (Thompson *et al.*, 1996). The only report of tristily in the Thymelaceae is in the species *Dais continifolia* L., a South African shrub to small tree (Barrett, 1993)

Oxalis

In the Oxalidaceae, tristily and distily are known in *Oxalis*, *Biophytum* and *Averrhoa* (Weller, 1992). *Oxalis* is a very large genus, including more than 800 species distributed globally, in tropical and cold regions, from sea level to the snow line in the highest mountains (Salter, 1944; Woodson *et al.*, 1980). The main centres of distribution are Central and South America and South Africa.

As in all tristylous species, *Oxalis* species also revealed deviations from the fully expressed system, as described in Darwin (1877), into a multifaceted system with many disruptions to the

expression of the different parts that compose the syndrome. Examples of such studies follow below in a short review.

In a comparative study between three subspecies of *Oxalis pricea* Small, ssp. *colorea* (Small) Eiten, ssp. *priceae* Small and ssp. *texana* (Small) Eiten, Mulcahy (1964) explored the lack of Mid morphs in the latter two subspecies. The author identified a possible relationship between clonal growth and its promotion of inbreeding as the probable reason for Mid morph extinction.

Trelease (1882) studied a large population of *Oxalis violacea* L. in Wisconsin, United States, that only included two morphs, Long and Short. The flowers of these morphs had two anther whorls and one stigma whorl, but instead of being arranged reciprocally, the stigmas in one morph were of intermediate length between the two sets of stamens of the other morph. Pollen grains sizes were also interesting, with both anthers of a morph containing pollen of nearly equal size, but of a different size to pollen from the other morph. He also observed that both flower types produced capsules, so both morphs were clearly still fertile.

O. dilenii Jacq. subsp. *filipes* (Small) Eiten and *O. priceae* Small. primarily produce homostylous populations, but both still contain dimorphic and trimorphic populations (Weller, 1992). In *O. stricta* L. var. *viridiflora* the plants studied showed no difference in organs heights, they were completely self-fertile and produced seeds in large quantities (Hus, 1907).

Ornduff (1964) investigated eight populations of *O. suksdorfii* Trel. of various ages in Oregon, United States, and showed all to be anisoplectic. He further showed that the Mid morph played a minor role as a source of seed.

Oxalis alpina (Rose) Knuth is known to have distylous and tristylous populations in southeastern Arizona, New Mexico and Mexico (Weller, 1978). Tristylous populations presented a variable frequency of Mid morphs, with mid morphs comprising between 1% to 46% of the total population (Weller, 1992). The incompatibility reactions in these populations were modified such that they resembled distylous species; Mid morphs are self-compatible and Long and Short morphs are inter-cross compatible when receiving pollen from a mid anther (Weller *et al.*, 2007).

Shibaïke *et al.* (1996) observed that *O. corniculata* L. in Japan is self-compatible and polymorphic in style length. Populations can be comprised of homostylous morphs, where heights of stigmas and longer stamens are matched, or long-styled morphs in which the stigmas are higher than the anthers. They found a relationship between environmental disturbance and the occurrence of these populations. Homostylous morphs more frequently occur in environments exposed to disturbance, while long-styled morphs are more frequent in coastal, less disturbed habitats.

There are well-known South African *Oxalis* species that colonize different countries in Europe and the Mediterranean region, Australia, California and South America (e.g. *O. pes-caprae* L. and *O. purpurea* L.) (Anna *et al.*, 2007; Platt *et al.*, 2005; Pütz, 1994; Rottenberg & Parker, 2004; Castro *et al.*, 2007). In all of these areas, populations of these invasive species are known to deviate from isoplethic equilibrium, often accompanied by altered incompatibility reactions. *O. pes-caprae* L. is the most common example, mostly containing Short morphs in these invasive populations, and plants are often pentaploid, reproducing only vegetatively (Pütz, 1994; Rottenberg & Parker, 2004; Castro *et al.*, 2007).

In South Africa, Salter (1944) also encountered anisoplethic populations. He often found *O. pes-caprae* in isolated populations comprised of a single morph. Cape Peninsula populations of *O.*

caprina L. all comprised of Short morph plants only and never produced any seeds. In contrast, populations of this species in the Caledon region (ca. 150 km away) had all three styler forms present and were producing seeds freely. Zietsman (2008) found in two endangered species. *O. comptonii* Salter and *O. oculifera* E.G.H.Oliv., that both produced seed after selfing. Similarly *O. purpurea*, a widespread species, presented deviations in the reciprocal arrangement of organs and self-incompatibility expression. Species found to have anisoplectic populations included *O. purpurea* and *O. tomentosa* L.f. (Zietsman, 2007).

As is obvious from this review, tristylous expression among American members of *Oxalis* has been fairly well-documented, while very little is known about the expression of tristylous among South African members of the genus. As tristylous expression was found to break down, especially among American members of *Oxalis*, deviations from full tristylous expression are also expected among South African members of the genus. Research presented here assesses the state of expression of the tristylous system among South African members of *Oxalis* in five different ways: 1. an assessment of the morph equilibrium in *Oxalis* populations; 2. an assessment of sexual reproductive organs reciprocity and ancillary characters; 3. a parallel analysis of patterns of tristylous expression between South African and American members of *Oxalis*, 4. a study of *Oxalis* section *Sagittatae* and 5. an assessment of the tristylous state and fertility of the polyploid taxon *O. hirta* var. *tubiflora*. They cover general aspects of the syndrome in the first two studies, while addressing specific aspects in the last three case studies. This research will contribute substantially to the growing understanding of *Oxalis* in South Africa with new insights into their sexual reproductive system, enabling future analogies with the phylogenetic study that is currently underway.

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1. Dissertation Structure

2. Tristyly in flowering plants:
A review

**3. Morph representation in
Oxalis populations across the
Cape Floristic Region**

4. Tristyly and sexual organ
morphology in South African
Oxalis

5. Patterns related to morph
frequency in tristylous *Oxalis*
species

6. Three-dimensional
reciprocity in *Oxalis*

7. Tristyly and self-
incompatibility in a polyploid
South African *Oxalis* species

8. Tristyly in the genus *Oxalis*
in South Africa: A review

**CHAPTER 3 - MORPH REPRESENTATION IN *OXALIS* POPULATIONS ACROSS THE CAPE
FLORISTIC REGION**

Summary

This study examined the state of tristily expression among South African members of *Oxalis*. Tristily has a large impact on fertilization success and ultimately on the equilibrium of a population. The sexual reproduction of South African *Oxalis* species has not yet been carefully investigated and species are generally considered to be tristylous. We considered tristily expression at species level including 58 different species and compared this between different populations. A total of 104 populations were studied in their natural habitats during the course of the 2005, 2006 and 2007 flowering seasons (April to August). Morph percentages of each population were calculated and morph frequency was compared by G-test for goodness-of-fit to isopletic equilibrium. Equal morph representation was not fully expressed in all populations. In total, 71 % of the populations were at isopletic equilibrium, while 29 % of populations deviated significantly from the expected ratio.

Key words: Tristily, *Oxalis*, population ratios, heterostyly, South Africa.

Introduction

In 1944, Salter published a revision of the genus *Oxalis* in South Africa, a result of 11 years of observations of living plants in their natural habitats. He focused on the taxonomy of the genus, and thus provided only brief notes on heterostyly and the expression thereof. He worked on the assumption that all South African plants were “probably trimorphic” (Salter, 1944).

Heterostyly is the occurrence of different forms of sexual organ arrangements in different plants of the same species (Darwin, 1877). In tristylous, a population is composed of three morphs, distinct from each other in the heights of stigmas that alternate with anthers reciprocally at three levels (Barrett, 1992). The three morphs, Long, Mid and Short, are expected to be equally represented in a population due to frequency-dependent mating, since legitimate inter-morph crosses are the only ones assumed to produce full seed set (Barrett *et al.*, 1997). The equal ratio of morphs is known as isopleth equilibrium (1:1:1) and deviations from it have been related to a number of reasons discussed below.

Tristylous in *Oxalis* has only been studied in single species or in isolated circumstances (Darwin, 1877; Trelease, 1882; Hus, 1907; Mulcahy, 1964; Ornduff, 1964; Weller, 1978, 1992; Shibaïke *et al.*, 1996; Rottenberg & Parker, 2004). The two *Oxalis* sections that received the most attention in terms of assessment of tristylous expression, *Corniculatae* and *Ionoxalis*, are native to the American continents. From these studies tristylous breakdown has been evident in both sections; the incompatibility system has not been maintained, morphological variations in sexual organs have been observed and populations range from biased to populations with complete absence of morphs. Due to the nature of the present study we will focus on the isopleth equilibrium aspect as an indication of the morphological state of tristylous in South African members of *Oxalis*.

Ornduff (1974) studied 15 populations of South African *Oxalis* species, and found most to be isoplectic, while some populations were Long-morph biased. Zietsman *et al.* (2007) found some populations at isoplectic equilibrium in *Oxalis purpurea* L., while other populations were anisoplectic, and some populations lacked one or even two morphs.

Currently there are 201 *Oxalis* species and *ca.* 270 taxa recognized in South Africa (Salter, 1944; Dreyer *et al.*, 2009). *Oxalis* species are concentrated along the southwest coast of Africa, from Namibia in the north, progressively increasing in number southward, with the Cape Floristic Region housing the greatest concentration of species (Oberlander *et al.*, 2002). The species are distributed in three main centres in this region, all presenting different environments in terms of soil composition and vegetation. South African species are all geophytes with true bulbs, and the bulb structure differs from that of the few American bulbous species (Salter, 1944; Oberlander *et al.*, 2009). The possession of underground storage organs has had a great impact on population dynamics in other heterostylous species, in that it enables shifts between vegetative and sexual reproduction according to changes in the environment, with clonal propagation reducing the strength of frequency dependent selection (Ornduff, 1964; Barrett, 1977; Weller, 1992; Eckert & Barrett, 1993).

Since the present state of the morphological expression of tristylous among diverse South African members of *Oxalis* is poorly known, we set out to determine if *Oxalis* populations in southern South Africa present any signs of morph variation from the expected tristylous equilibrium in populations and if so, to investigate if these variations occur in the same manner within a species. The next aim was to determine if the lack of morph equilibrium presents any relationship with environmental parameters such as disturbance, vegetation type and structure, among different populations of the same species.

Material and Methods

The morphological expression of tristylly was evaluated through measurement of population morph frequency ratios in 58 species of *Oxalis*, studied in their natural habitats during the flowering seasons of 2005, 2006 and 2007. For 17 of these species, more than one population was analyzed, bringing the total number of populations studied to 104. Species were selected to adequately represent each of the main clades identified in the molecular phylogeny of southern African *Oxalis* (Oberlander *et al.*, 2009).

Population localities were recorded using a GPS system, and each site was graded according to the degree of disturbance. There were five disturbance categories ranging from highly disturbed to pristine. Factors that influenced this ranking included exact locality (edge of roads, farmland, natural) and ecological aspects such as evidence of recent fires, vegetation type and vegetation structure. Other characters noted included elevation, substrate and soil moisture capacity (Appendix 1). Each species was identified by using the keys and descriptions in Salter (1944), and this was verified by comparisons to collections in the Bolus Herbarium (University of Cape Town, South Africa) and the living collection in the Stellenbosch University Botanical Gardens.

The number of flowers collected for study differed according to the size of the population. In populations with more than 100 plants, 100 plants were sampled per species per population, while in smaller populations 50 plants were sampled per species. The great majority of species produce single-flowered peduncles; for those with multiple flowers only one flower was sampled per inflorescence per plant. In all populations the flowers were collected randomly at 2-meter intervals to minimize potential effects of clonality (Salter, 1944). The flowers were kept fresh in Ziploc plastic bags, and when not analyzed on the day of collection, the bags were refrigerated for no longer than two days.

The morph type of each flower was determined visually (naked eye) after removal of petals and sepals, and for each population the percentage of each morph type was calculated. For all species, morph frequencies were compared by G-test for goodness-of-fit (Sokal & Rohlf, 1995) to an isoplethic (1:1:1) equilibrium, and rated as being in equilibrium or not at 95% and 99% significance levels, with cut off threshold of $G > 5.99$ and $G > 9.21$, respectively. Significance at both these levels was considered as an indication of the degree of deviation from the expected isoplethic ratio. For species where more than one population was collected, pooled goodness-of-fit tests were conducted to determine if there was a consistent trend in the data. G- tests of heterogeneity were also performed to determine the levels of heterogeneity among populations, while the G-total was used as an indication of the overall measure of departure from isoplethy of all the populations surveyed together.

Results

Equal morph representation was not consistently found in all populations studied (Table 3.1). Thirty populations, representing 28.8 % of the total number of populations, were anisoplethic (Fig. 3.1). These populations deviated significantly from the expected equilibrium of morphs (1:1:1) by either having one morph that dominates significantly, one morph with significantly fewer individuals, or displaying the lack of one or even two entire morphs within the population sample.

Shifts in morph representation varied both among and within the species (Table 3.2). Six species had dimorphic populations and two species displayed monomorphic populations. Within a species, there were some populations at isoplethic equilibrium, while others were anisoplethic, with the latter varying in terms of morph representation and proportion. The only exception to this was found in *Oxalis incarnata* L, presenting a departure from isoplethy in both populations examined. This species had one dimorphic population and one monomorphic population, and in

Table 3.1: Summary of morph percentages recorded in populations of *Oxalis* species.

Percentages of Long (L), Mid (M) and Short (S) morphs are based on the total number of flowers analyzed (N). Significant deviation from isoplethic equilibrium was determined through G-statistics. G is the likelihood ratio between sampled and expected frequencies.

Identification	Pop. code	% L	% M	% S	N	Deviation from IE	G
<i>O. adspersa</i>	6	33.0	21.7	45.3	106	*	9.00
<i>O. callosa</i>	68	25.3	45.5	29.3	99	*	6.54
<i>O. caprina</i>	2	0.0	100.0	0.0	47	**	103.26
<i>O. caprina</i>	10	0.8	0.0	99.2	124	**	260.82
<i>O. cf. fergusoniae</i>	87	0.0	6.3	93.7	79	**	136.30
<i>O. cf. gracilipes</i>	50	36.8	43.2	20.0	95	*	8.78
<i>O. ciliaris</i>	75	48.5	31.7	19.8	101	**	12.70
<i>O. comptonii</i>	27	27.5	21.6	51.0	51	*	7.08
<i>O. densa</i>	54	33.0	23.0	44.0	100	*	6.70
<i>O. dregei</i>	59	30.6	17.1	52.3	111	**	21.07
<i>O. glabra</i>	3	0.0	83.9	16.1	31	**	40.72
<i>O. glabra</i>	52	58.7	26.9	14.4	104	**	31.85
<i>O. hirta</i>	32	27.6	23.7	48.7	76	*	7.85
<i>O. hirta</i> var. <i>tenuicaulis</i>	104	26.7	26.7	46.5	101	*	7.53
<i>O. hirta</i> var. <i>tubiflora</i>	28	33.3	44.4	22.2	90	*	6.80
<i>O. incarnata</i>	66	65.3	34.7	0.0	101	**	91.57
<i>O. incarnata</i>	90	100.0	0.0	0.0	25	**	54.93
<i>O. lanata</i>	63	6.7	0.0	93.3	90	**	153.66
<i>O. massoniana</i>	67	35.9	40.0	24.1	145	*	6.16
<i>O. nidulans</i>	98	0.0	3.0	97.0	165	**	317.73
<i>O. nidulans</i> var. <i>denticulata</i>	31	35.0	44.0	21.0	100	*	8.44
<i>O. oculifera</i>	26	25.7	46.8	27.5	109	*	8.51
<i>O. pes-caprae</i>	7	24.3	12.6	63.1	111	**	45.03

<i>O. pes-caprae</i>	57	28.6	46.2	25.2	119	*	8.71
<i>O. polyphylla</i>	77	25.7	28.4	45.9	109	*	7.50
<i>O. purpurea</i> (pink)	47	1.0	6.9	92.2	102	**	162.00
<i>O. recticaulis</i>	82	66.7	18.8	14.6	48	**	22.43
<i>O. sp.</i>	72	21.6	38.2	40.2	102	*	6.90
<i>O. tomentosa</i>	84	25.5	70.9	3.6	55	**	42.46
<i>O. zeekoevleyensis</i>	9	44.0	44.0	12.0	100	**	24.34

* = $P < 0.05$ and ** = $P < 0.01$

Figure 3.1: Anisoplethic populations. The population code is provided in parenthesis, values in the bars represent the percentage of occurrence of Long (horizontal lines), Mid (blank spaces) and Short (dots) morphs and are indicated by numbers.

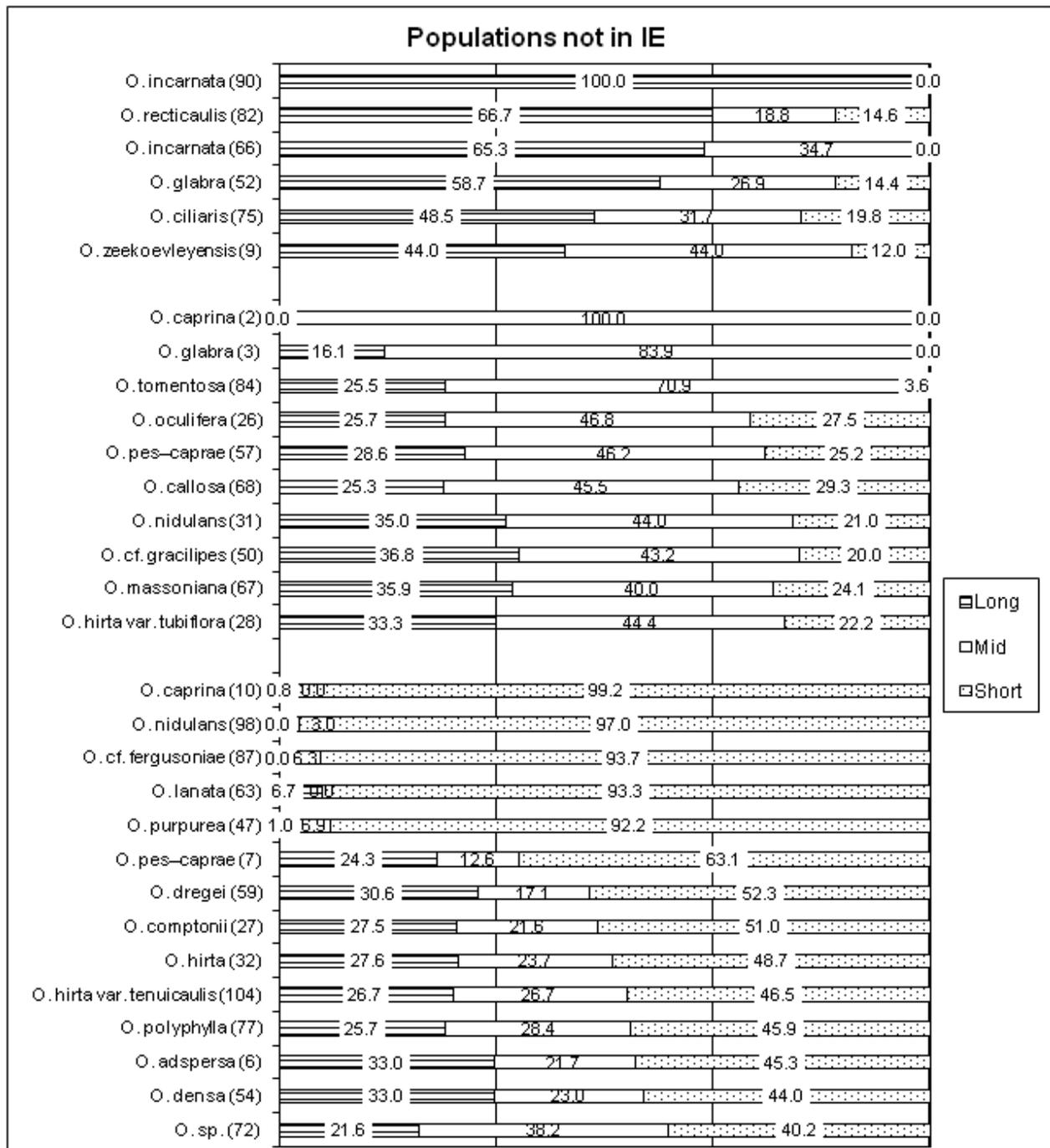


Table 3.2: Summary of species with more than one population sampled (n) showing the levels of heterogeneity among the populations per species, the measure of consistency of the data and the overall measure of departure from isopleth of populations per species. G-het is the ratio of homogeneity of replicates, G-pooled is a measure of significant trend, G-total is the sum of G-het and G-pooled; all according to degrees of freedom (df) and the significance compared with χ^2 (p).

Identification	n	Ghet	df	p	Gpooled	df	p	Gtotal	df	p
<i>O. adspersa</i>	2	2.49	2	NS	10.20	2	**	12.69	4	*
<i>O. caprina</i>	2	201.10	2	**	162.99	2	**	364.08	4	**
<i>O. ciliaris</i>	3	13.28	4	**	6.15	2	*	19.44	6	**
<i>O. depressa</i>	2	1.68	2	NS	1.40	2	NS	3.08	4	NS
<i>O. glabra</i>	6	70.33	10	**	13.94	2	**	84.27	12	**
<i>O. hirta</i>	3	6.82	4	NS	3.09	2	NS	9.91	6	NS
<i>O. hirta</i> var. <i>tubiflora</i>	3	7.41	4	NS	1.78	2	NS	9.19	6	NS
<i>O. incarnata</i>	2	18.54	2	**	127.95	2	**	146.50	4	**
<i>O. natans</i>	2	0.52	2	NS	5.27	2	NS	5.79	4	NS
<i>O. nidulans</i>	6	281.75	10	**	47.45	2	**	329.20	12	**
<i>O. obtusa</i>	3	2.50	4	NS	4.69	2	NS	7.19	6	NS
<i>O. pes-caprae</i>	5	62.46	8	**	1.71	2	NS	64.17	10	**
<i>O. purpurea</i>	8	171.01	14	**	6.48	2	*	177.48	16	**
<i>O. suteroides</i>	2	1.64	2	NS	0.82	2	NS	2.46	4	NS
<i>O. tenella</i>	2	1.66	2	NS	2.08	2	NS	3.73	4	NS
<i>O. tenuifolia</i>	2	0.18	2	NS	4.51	2	NS	4.69	4	NS
<i>O. tomentosa</i>	3	33.08	4	**	15.90	2	**	48.99	6	**

NS = not significant, * = $P < 0.05$ and ** = $P < 0.01$

both cases the Long morph dominated and the Short morph was absent, causing them only to vary in terms of Mid morph frequency.

In seventy-four populations (71.2 %) the three morphs were at isoplethic equilibrium. Populations in equilibrium were found to grow in different regions, substrates, vegetation types, exposure and levels of disturbance (Appendix 1).

Discussion

Tristyly breakdown has been previously attributed to a variety of factors (Barrett, 1992). Here we focused on geographical location and degree of clonality, two suggested reasons that have been indicated in previous studies of *Oxalis* to influence tristyly breakdown (Weller *et al.*, 2007; Zietsman, 2007).

The influence of geographical location on morph ratios was assessed in American populations of *O. alpina* (Rose) Knuth, where variation in Mid morph frequencies were related to ecology (Weller, 1992; Weller *et al.*, 2007). In another case, Shibaike *et al.* (1996) observed that in Japan *O. corniculata* L. had homostylous and Long morph biased populations, which coincided with two very different localities, one in rural areas and the other near the coast. In South Africa, Zietsman (2007) observed a geographical trend in the pattern of tristyly expression of *O. purpurea*, showing that the anisoplethic populations were exclusively restricted to the eastern periphery of the distribution range of this species.

At first glance our results suggest that *Oxalis* species that display anisoplethy in South Africa are not limited to specific areas or environments. Isoplethic and anisoplethic populations very often grow sympatrically, and in such populations it cannot be assumed that environmental parameters cause such deviations of tristyly morph expression in all species. Our results also showed that

species for which more than one population were sampled, and which showed signs of isopleth deviation, are not restricted to specific geographical areas. *O. ciliaris* Jacq., *O. glabra* Thunb., *O. hirta* L., *O. hirta* L. var. *tubiflora* (Jacq.) Salter *O. nidulans* E. & Z. and *O. pes-caprae* L. all have anisoplethic populations that grow in different geographical areas, with no obvious geographical boundaries or barriers associated with these deviations in tristylous expression, contrasting to results of Zietsman (2007).

Although our results do not show geographic location to directly influence the equilibrium of morphs, our observations suggest that the morph expression observed in *Oxalis* populations in South Africa are driven by small-scale ecological conditions rather than a broad geographical pattern. The Western Cape Province is topographically diverse due to geographic and altitudinal variation. The mountains provide a range of small habitats with distinctly different micro-climates, resulting in many diverse micro-environments present in relatively close geographical proximity (Goldblatt & Manning, 2000; Linder, 2003). Individual populations are often restricted to very specific soil types, moisture regimes or surrounding vegetation matrices. The uniqueness of ecological conditions (from arid to aquatic) in which *Oxalis* species grow should influence their mode of reproduction, exerting different pressures on seed production and establishment, directly affecting the rate of vegetative and sexual reproduction.

It has been shown that aspects of the sexual system such as flower formation and seed production can be slowed down by high rates of clonal propagation (Eckert & Mavragnis, 1996). Ornduff (1964) showed that populations of *O. suksdorfii* Trel. are anisoplethic, in part, due to sporadic sexual reproduction and dominant vegetative reproduction, distylous in *O. priceae* Small. spp. *priceae* was related to clonality, homostylous populations of *O. corniculata* were probably established from few phenotypes and finally loss of the Mid morph in *O. alpina* were

related to a combination of factors including vegetative reproduction (Shibaike *et al.*, 1996; Weller, 1981a, 1981b, 1992).

All South African *Oxalis* species can reproduce clonally through bulbil formation, but levels of clonality differ between species, with some species propagating through bulbil formation to the extent that they have become noxious international weeds (Pütz, 1994; Rottenberg & Parker, 2004; Platt *et al.*, 2005; Sala *et al.*, 2007; Castro *et al.*, 2007). Among the species with anisoplethic populations are some that formed clonal clumps, such as in *O. hirta* var. *tubiflora* (Gifberg Mountains population) and *O. tomentosa* L. f. (Darling population), while others never formed such clonal clusters, for example *O. nidulans* (Jonaskop Nature Reserve population). In this population, almost all plants were of the Short morph, but individual plants were scattered 2 to 5 meters apart. Among isoplethic populations, there were also species with high levels of clonality, such as *O. hirta* (Darling population) and *O. purpurea* (Stellenbosch population). These examples obscure the influence that clonal propagation may have on the equilibrium of morphs in populations and in species.

The grand average for anisoplethic populations does not indicate a bias towards a specific morph. Therefore, the lack of a general pattern suggests that stochastic forces are the most likely cause of the deviations from isoplethy. Stochastic forces are events that alter the frequency of morphs in populations mostly in a random way. This may include, among others, founder events (such as migration, long distance dispersal, or local colonization) and genetic drift (influencing the frequency of alleles) (Barrett, 1993). Because of the particular mode of inheritance of tristylly, frequency dependent selection provides the dominant force maintaining floral trimorphism, while stochastic forces have the potential to destabilize the polymorphism (Barrett, 1993). Alterations in self-incompatibility, changes in the reciprocity of organs, as well as perennially and clonal propagation, constrain the rate of recruitment of offspring resulting from

disassortative mating and allow stochastic forces to have a greater influence on morph frequencies (Barrett, 1993). Ornduff (1972, 1974) observed these to be acting in *Oxalis* Section *Corniculata* and in other *Oxalis* species in South Africa.

In order to understand the expression of the tristylous system in these species, it will be necessary to investigate the environmental conditions within each particular area for a given population and relate these parameters to optimal sexual reproduction, providing then, grounds for comparison among populations of the same species. The ranges of specific species should be evaluated in more detailed case studies in order to formulate a general rule or pattern of environmental impact on the balance of morphs in populations.

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1. Dissertation Structure

2. Tristyly in flowering plants:
A review

3. Morph representation in
Oxalis populations across the
Cape Floristic Region

**4. Tristyly and sexual organ
morphology in South African
*Oxalis***

5. Patterns related to morph
frequency in tristylous *Oxalis*
species

6. Three-dimensional
reciprocity in *Oxalis*

7. Tristyly and self-
incompatibility in a polyploid
South African *Oxalis* species

8. Tristyly in the genus *Oxalis*
in South Africa: A review

Summary

The study of different aspects of floral morphology in heterostylous species has been widely used as a tool to understand the pollination process, as it significantly influences pollen transfer and reproduction. Here, we study sexual organ morphology in South African *Oxalis* species, one of the few genera to express tristily in most of its large number of species. The heights of sexual reproductive organs were measured in all morphs of 104 populations comprising 58 species. Lloyd's ratio of organ heights and reciprocity index values were calculated for these populations to determine the consistency of organ separation. Also, pollen grain sizes of all anther levels and all morphs were compared for 15 species. Most populations had smaller Lloyd's ratios of organ heights (a measure of average distance between organ levels) than in recognized tristylous species. However, Lloyd's reciprocity index values confirmed clear separation between organ levels. Differences in pollen grain sizes were, for the most part, linked to differences in anther heights. No general relationship was identified between the ratios of average heights, reciprocity indexes and anisoplety. Similarly, differences in pollen grain sizes could not always be explained by anisoplety.

Key words: *Oxalis*; pollen morphology, reciprocity; sexual organ arrangement; South Africa.

Introduction

Plant sexual reproductive systems display diverse structural and temporal combinations of male and female organs with important functional consequences for fitness and mating behaviour (Barrett, 2002). Heterostyly is defined by the structural variation of style and stamen lengths, including tristylous and distylous systems. Tristylous species have three different floral morphs in the same population, while distylous species have only two floral morphs (Darwin, 1877). In a tristylous species each morph has two separate whorls of stamens and a single stigmatic whorl, arranged reciprocally such that anthers and stigmas alternate in height (reciprocal herkogamy) (Ganders, 1979).

Lloyd *et al.* (1990) and Lloyd and Webb (1992) defined heterostyly by the presence of reciprocal herkogamy alone, with heights corresponding more or less between the morphs. Their interpretation was based on definitions by Darwin (1877) and Ganders (1979), while recognizing that organs frequently do not match in height. They further argued that tristily requires the two anther levels to be well separated, and adopted the concept of relative position of pollinating surfaces, rather than absolute stamen and style lengths. Thus, the position of anthers and stigmas distinguish the morphs from each other, and reciprocal herkogamy does not necessarily imply that organs must be at the same height, but rather that the variation and sequence of heights is preserved. Essentially, this is what differentiates heterostyly from the arrangement in any other polymorphism, and therefore their definition is followed in this paper.

Morphological and physiological components of heterostyly have different functional roles in promoting male and female fitness (Barrett *et al.*, 2000). Small differences in the positioning of stigmas and anthers can significantly change the rate of disassortative pollination (Ganders, 1979). Characteristics such as the position of anthers and stigmas, the amount of pollen produced and differences in pollen grain size have also been identified as being valuable in

regulating disassortative pollen flow (Dulberger, 1992; Barrett *et al.*, 2000). These differences represent possible advantages for certain morphs, compromising sexual reproduction and the equilibrium of morphs in populations (Medrano *et al.*, 2005; Rossi *et al.*, 2005). Breakdown of the tristylous system has been observed in many species from different families (Weller, 1992; Eckert & Mavraganis, 1996; Barrett *et al.*, 1997). Examples of this, including failure on the fertilization process, have also been recorded among American members of *Oxalis* (Trelease, 1882; Hus, 1907; Ornduff, 1964, 1972). Recorded cases of morphological deviation among South African taxa are discussed by Zietsman *et al.* (2007) but they are limited.

In this paper we address the state of sexual organ morphology in South African *Oxalis* populations. The main goal was to identify if sexual organ arrangement is fully tristylous by analyzing their heights, positioning and reciprocity within and between morphs of the same population. Secondly, we aim to investigate the state of pollen grain sizes produced by anthers of different heights of different morphs within a population, addressing the tristylous state of this ancillary character. Finally, we want to investigate if morphological expression in these two instances had any relation to variation in morph equilibrium on the populations studied.

Materials and Methods

Sexual organs

Sexual reproductive organs were studied in 104 populations of 58 *Oxalis* species (Appendix 2). The heights of male and female reproductive organs of 20 flowers per morph per population were measured using digital calipers (Toolquip - CE, 0-150 mm – Maitland, Cape Town, South Africa). Flowers were collected from individual plants at more than 2 meters apart from each other to avoid sampling cloned plants (Salter, 1944). All measurements were taken from the base of the ovary to the highest point of the relevant organ measured. To verify the degree of reciprocity between morphs, two indexes were calculated according to methods proposed by

Lloyd *et al.* (1990) (Fig. 4.1). Both indexes were calculated individually for each population. The first index, ratio of organ heights, was calculated by taking the average height of each level combining anthers and stigmas, resulting in three values (upper, mid and lower). The average mid-level and upper-level heights were then divided by the average lower-level height to obtain a ratio. This ratio quantifies the consistency of levels in all three morphs and the separation between them across a population. The second index, reciprocity index, was calculated by taking the average values for each level, separating anthers and stigmas, resulting in nine values. The differences between adjacent levels within a morph were calculated (Fig 4.1, a values). The differences within same levels among morphs were calculated (Fig 4.1, b values). We then divided the average distance between adjacent levels by the average difference within levels ($a \div b$), to determine the uniformity of the segregation between levels in each population. Higher reciprocity is indicated by larger values, while the inverse is also true. These same parameters have been calculated and used in studies of American members of *Oxalis* (e.g. *Oxalis tuberosa* Mol., *O. alpina* (Rose) Knuth, *O. suksdorfii* Trel. and *O. squamata* Zucc.) and in *Narcissus triandrus* L. (Lloyd *et al.*, 1990; Marco and Arroyo, 1998).

The indexes used in the present study were chosen among others due to their dynamic characteristics, including the natural variation in reproductive organ heights that occurs in a population and its application to tristylous populations (Eiten, 1963; Richards & Koptur, 1993; Eckert & Barrett, 1994; Sanchez *et al.*, 2008).

The results obtained in this analysis were compared with morph frequencies of each population (Appendix 2) in order to test if these two parameters present any relationship. The morph type of each flower was determined visually (naked eye) after removal of petals and sepals, and for each population the percentage of each morph type was calculated. For all species, morph frequencies were compared by G-test for goodness-of-fit (Sokal & Rohlf, 1995) to an isoplethic

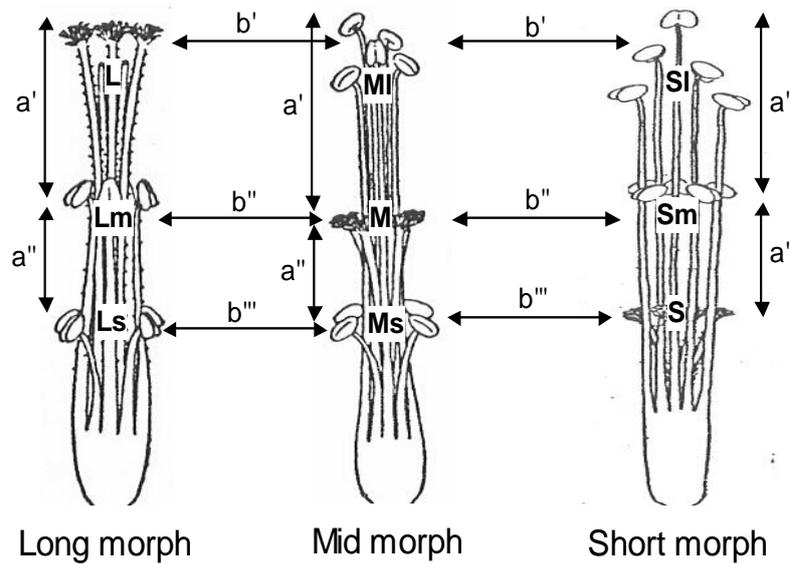


Figure 4.1: Diagrammatic summary of the calculations of reciprocity estimates. Stigmas are indicated by capital letters (L, M, and S) and define the three flower morph types (Long, Mid, and Short). Lower case letters (l, m, and s) indicate different stamen levels. Two distances between adjacent levels are found within a flower (a' and a'') in each morph. There are 3x3 pair-wise comparisons for differences of heights among levels between the morphs (b' , b'' and b'''). Redrawn using Salter's (1944) illustrations and following the Lloyd *et al.* (1990) model.

(1:1:1) equilibrium, and rated as being in equilibrium or not at 95% and 99% significance levels, with cut off threshold of $G > 5.99$ and $G > 9.21$ respectively. Significance at both these levels was considered as an indication of the degree of deviation from the expected isoplethic ratio.

Pollen morphology

Twelve species were studied with a Nikon Eclipse-E400 microscope (Nikon Instruments Europe B.V., Amstelveen, Netherlands) at 40X magnification and measured using a micrometer scale attached to the eyepiece. For two of these species, more than one population was analyzed. We thus collected pollen grain measurements for a total of 15 populations. Twenty pollen grains of each anther level, sampled from five different flowers per morph, were studied in every population. In total 100 pollen grains were analyzed per anther level per morph per population. Pollen grain measurements were analyzed using Kruskal-Wallis ANOVA by ranks (Statistica 8, StatSoft Southern Africa – Research (Pty) Ltd., Johannesburg, South Africa). Significance between subgroups (flower morph and organ height) was evaluated by Kruskal-Wallis multiple-comparison z-value tests.

Results

Sexual organs

Lloyd's ratio of organ heights and reciprocity index values for all populations analyzed are summarized in Appendix 2. Graphic illustrations of these indexes are shown in Figs. 4.2 and 4.3, in which five populations were chosen to illustrate extremes and central values of ratio of organ heights, reciprocity indexes and stigma and anther height arrangements (Fig. 4.4).

Lloyd's reciprocity index values ranged from 3.6 (*O. glabra* Thunb.) to 40.7 (*O. pes-caprae* L.) (Fig. 4.2 B and E, respectively). The reciprocity index values were generally lower in anisoplethic populations (Fig. 4.2 and Appendix 2), but this was not constant. In populations of

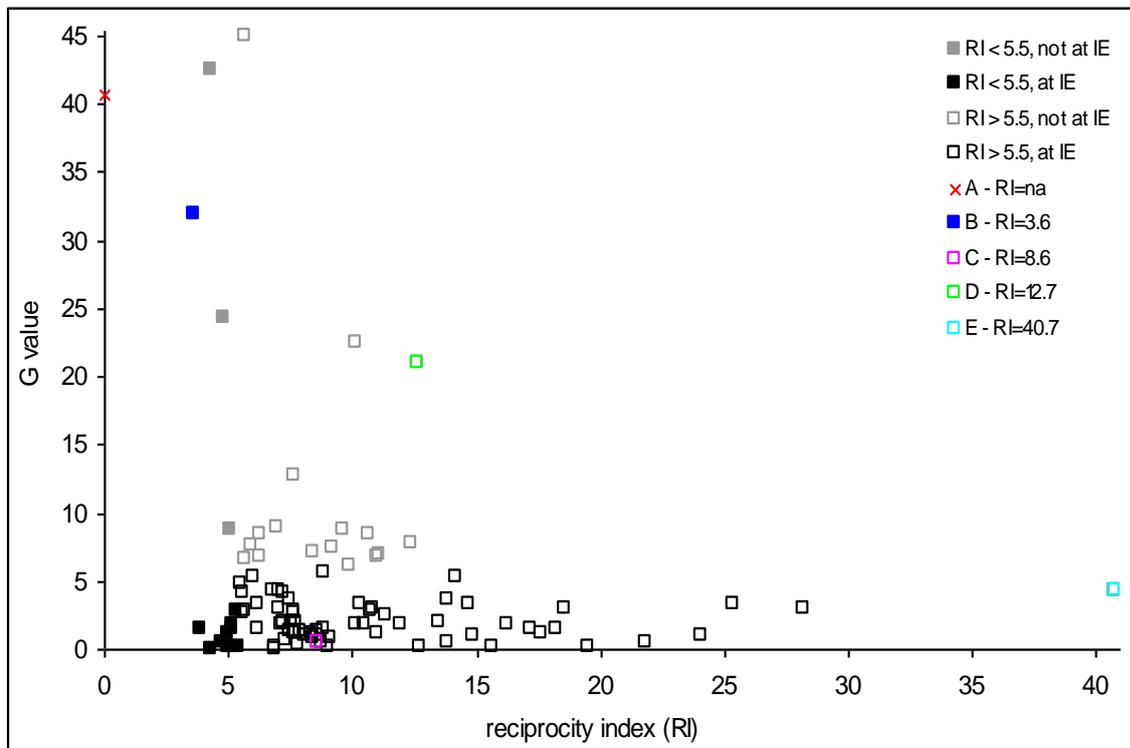


Figure 4.2. Scatter plot of data obtained from the calculations of the reciprocity index (RI) and their relation to G-values (an indication of population isopleth). Individual populations are represented by squares. Grey-coloured squares are populations not at isopleth (not at IE) and black-coloured squares indicate populations at isopleth equilibrium (at IE). Filled squares are populations with a lower reciprocity index than normally accepted for tristylous species (RI < 5.5) and outlined squares are populations with reciprocity index values within the range recognized for tristylous species (RI > 5.5). A to E represent populations used as examples in graphic comparisons (Figure 4.2 to 4.4). A: *Oxalis glabra* (population 3, RI = na), B: *O. glabra* (population 52, RI = 3.6), C: *O. aurea* (population 16, RI = 8.6), D: *O. dregei* (population 59, RI = 12.7), E: *O. pes-caprae* (population 37, RI = 40.7).

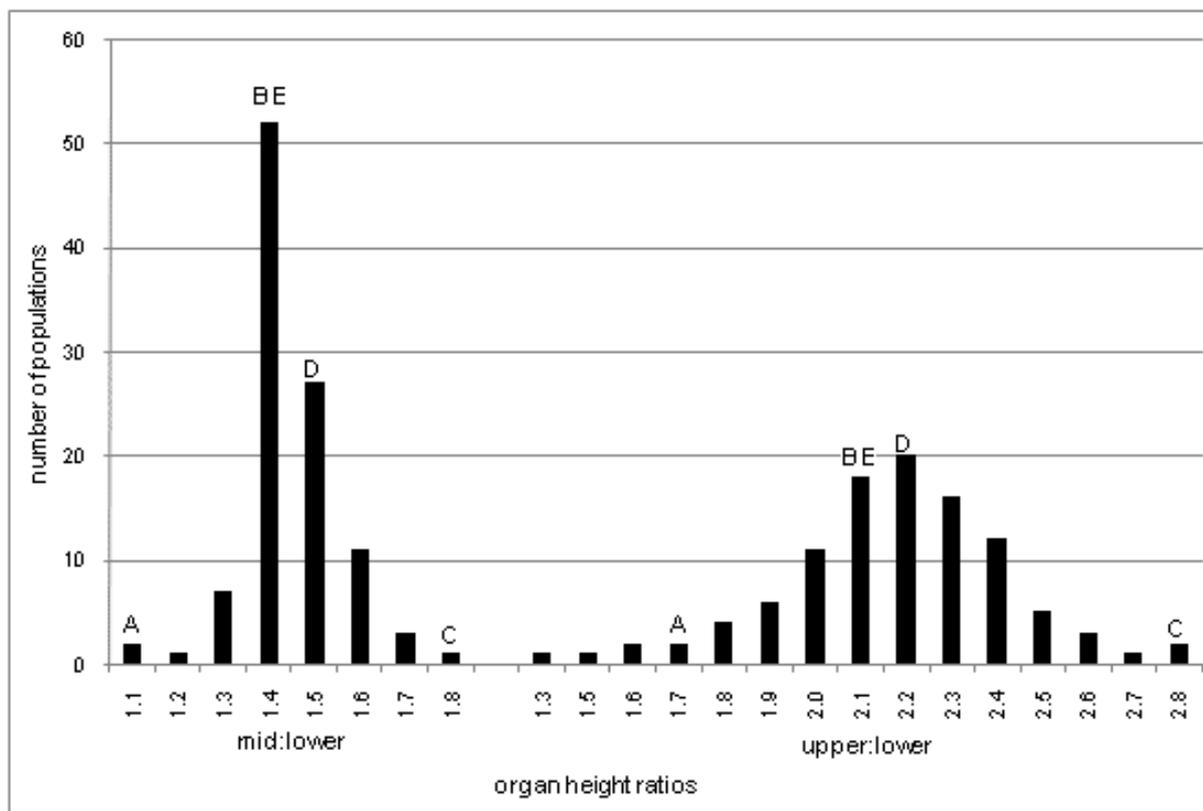
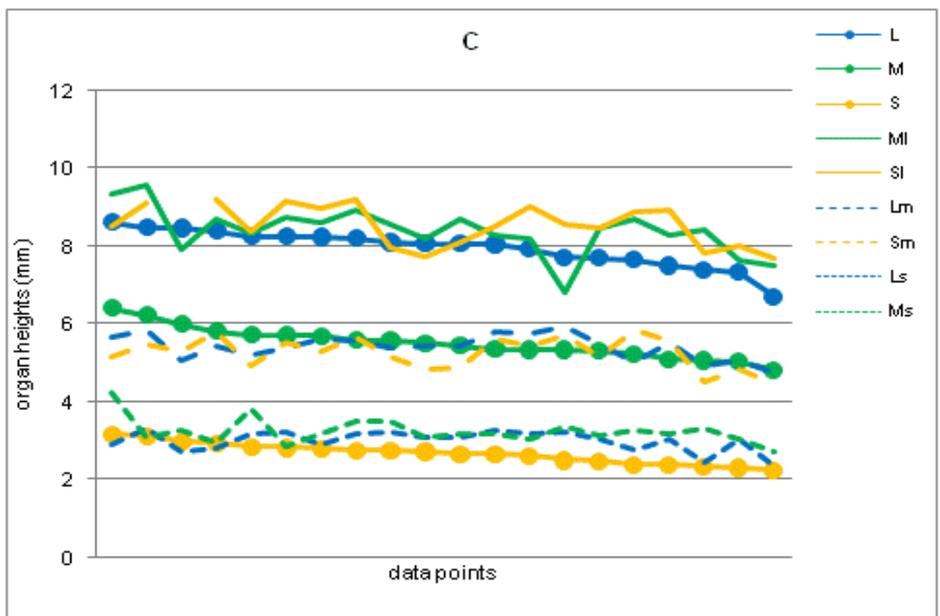
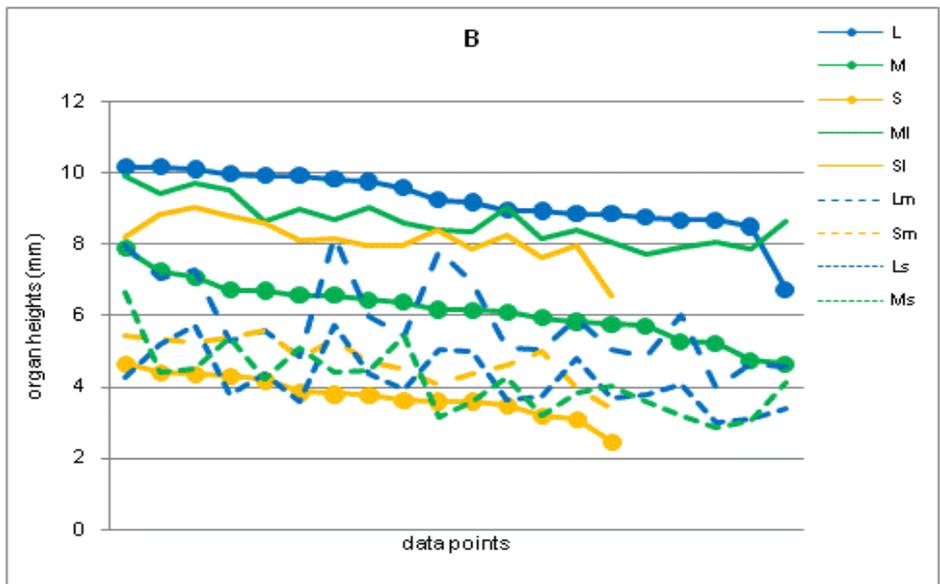
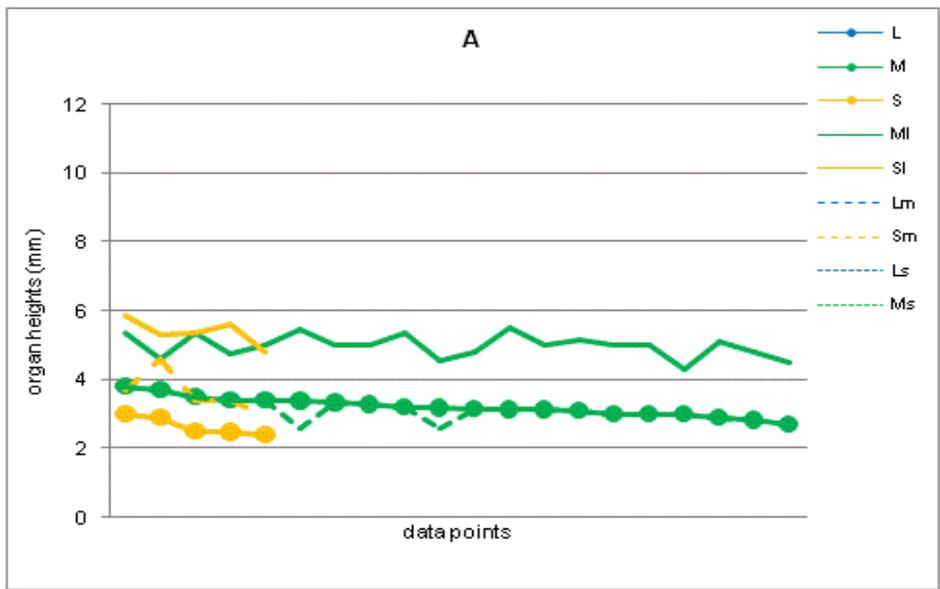


Figure 4.3. Frequency in number of populations (Y axis) in which a given ratio of organ height (X axis) occurred. Organ height ratios are given as ratio between short level organs:mid level organs (s:m) and short level organs:long level organs (s:l). Letters A to E represent populations used as examples in graphic comparison (Figure 4.2 to 4.4). A: *Oxalis glabra* (population 3, 1:1.1:1.7), B: *O. glabra* (population 52, 1:1.4:2.1), C: *O. aurea* (population 16, 1:1.8:2.8), D: *O. dregei* (population 59, 1:1.5:2.2), E: *O. pes-caprae* (population 37, 1:1.4:2.1).



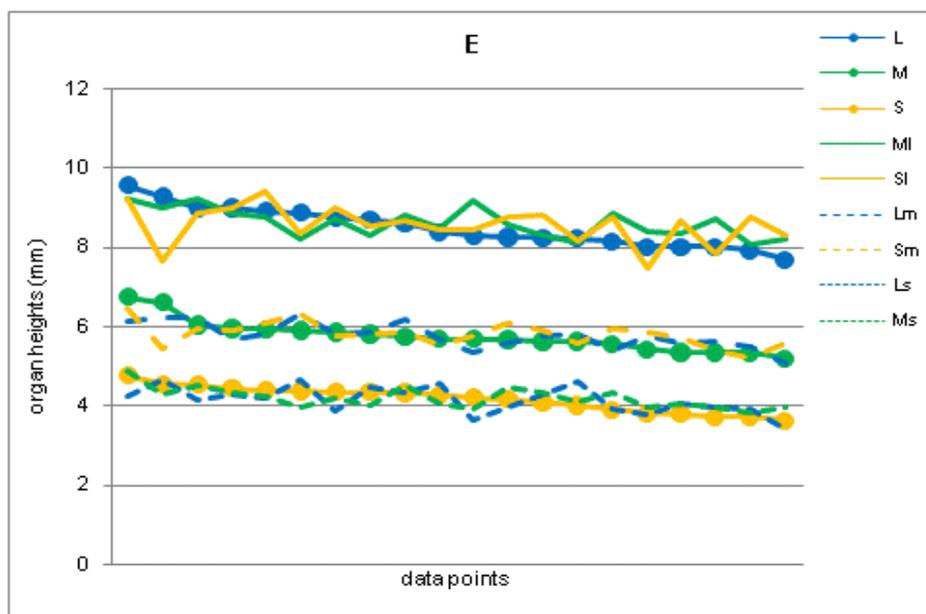
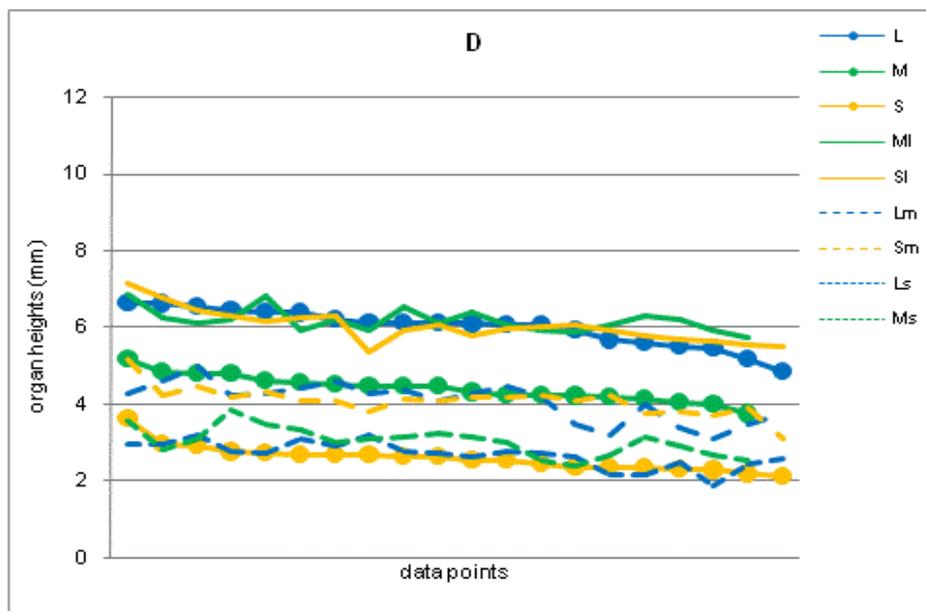


Figure 4.4. Graphical illustrations of stigmas and anther heights for five species of *Oxalis*. Graph A: *Oxalis glabra* (population 3), graph B: *O. glabra* (population 52), graph C: *O. aurea* (population 16), graph D: *O. dregei* (population 59), graph E: *O. pes-caprae* (population 37). The 20 data points for each organ level for all morph types are plotted along the X axis. Different colours represent organs of the different morphs: blue-Long, green-Mid and yellow-Short. Solid lines with dots represent stigmas (L, M, S) and are arranged in order of decreasing heights. Anthers (Lm, Ls, Ml, Ms, Sl, Sm) are represented by broken lines. Morphs are represented by capital letters L-Long, M-Mid, S-Short and are followed by small letters that represent the organ level, l-long, m-mid, s-short.

O. hirta L., *O. hirta* var. *tubiflora* (Jacq.) Salter and *O. ciliaris* Jacq., for example, the index remained the same in both anisopletic and isopletic populations. In some extreme cases anisopletic populations had reciprocity index values of more than 10 (e.g. *O. dregei* Sond., Fig. 4.2, D).

Most *Oxalis* populations studied (59%) have organ height ratios below the range normally recognized in other tristylous genera (smaller than 1:1.5:2.0), but similar to those found in American *Oxalis* species (Marco & Arroyo, 1998, Lloyd *et al.*, 1990). The ratio of organs heights varied from 1:1.1:1.3 (*O. nidulans* E. & Z.) to 1:1.8:2.8 (*O. aurea* Schltr, Fig. 4.3, C) and remained constant between populations of the same species, except in *O. nidulans* and *O. glabra* Thunb. In *O. nidulans*, the ratio of average heights ranged from 1:1.1:1.3 to 1:1.4:1.8, while in *O. glabra* the only change in ratios appeared in the anisopletic population (population 3, Fig. 4.3A).

Figure 4.4A represents *O. glabra* (population 3) which lacks Long morphs, rendering it impossible to calculate the reciprocity index of this population. The value for the ratio of average heights reflects that in this population; Mid morph stigmas (M) and the lower anther level (Ms) are of the same height. Reciprocity indexes were similarly impossible to calculate in eight further populations (population 2, 3, 10, 63, 66, 87, 90 and 98) representing the following 6 species: *O. caprina* L., *O. glabra*, *O. incarnata* L., *O. lanata* L., *O. nidulans* and *O. cf. fergusoniae*. Figure 4.4B represents *O. glabra* (population 52) in which all three morphs were present, but not at isopletic equilibrium. Although the ratio of average heights (1:1.4:2.1) in this population was higher than that of another population of the same species (population 3), showing some degree of separation of levels, the reciprocity index was low (3.6) as organs were not uniformly arranged.

Figure 4.4C represents *O. aurea* (isopleletic population 16) with an average reciprocity index of 8.6, and the highest ratio of organ heights (1:1.8:2.8) measured. Figure 4.4D shows *O. dregei* (population 59) with an intermediate ratio of average heights (1:1.5:2.2) and a higher reciprocity index value (12.7). This population was anisopleletic, with all three morphs present, but with Mid morphs present at a very low frequency (17.1%), while Short morphs were present at a high frequency (59%). Figure 4.4E shows a population of *O. pes-caprae* (population 37) with the same ratio of average heights as *O. glabra* (population 52), but with the highest reciprocity index (40.7) of all *Oxalis* populations measured.

In contrast to the arrangement expected in tristylous species where sexual organs appear at three distinct heights, many *Oxalis* populations in South Africa clearly had more than strictly three levels. There were some cases in which the 5 long-level anthers of Mid and Short morph flowers formed a ladder cascading down between the long-level and mid-level positions, while the mid-level organs remained of equal length. This arrangement can be seen in Figure 4.1 in the upper level anthers of the Short morph. This arrangement was found in *O. tenella* E. & Z. (population 17), *O. multicaulis* E. & Z. (population 79) and *O. stellata* E. & Z. (population 91). In *O. hirta* (population 32), *O. campicola* Salter (population 40), *O. xantha* Salter (population 44), *O. sp.* (population 73), *O. glabra* (population 80), *O. recticaulis* Sond. (population 82), *O. nidulans* (population 83), *O. ciliaris* (population 94) and *O. sp.* (population 99) individual long level anthers decreased in height to such an extent that they were at the same height as mid level organs. The anthers and stigmas of most species studied did not extend beyond the corolla tube, with the notable exception of *O. hirta* var. *tubiflora* in which long stigmas exerted way beyond the corolla even prior to anthesis.

Pollen morphology

In eight of the 15 populations analyzed, variation in pollen grain sizes followed variation in

anther heights; long level anthers produced the largest pollen grains, short level anthers produced the smallest pollen grains, and mid level anthers produced pollen grains of an intermediate size (Table 4.1). *O. cf. fergusoniae* (population 87), *O. sp.* (population 99), *O. incarnata* (population 90), *O. nidulans* (population 98), *O. hirta* var. *tubiflora* (population 21) and *O. hirta* var. *tubiflora* (population 28) produced pollen grains of sizes that followed anther levels, but with no significant size differences in pollen grains sampled from different morphs. In *O. adspersa* E.& Z. (population 6) and *O. incarnata* L. (population 66), however, short level anthers of different morphs produced significantly different sized pollen grains. Short level anthers of the Mid morph produced larger grains than short level anthers of the Long morph.

The remaining seven populations showed marked variation in pollen grain sizes. In *O. hirta* var. *tubiflora* (population 15) both anther levels of the Long morph produced smaller pollen grains than those of correspondent levels in other morphs; mid level pollen in this morph (Lm) was no different from short level pollen from the Mid morph (Ms), and pollen from the short level anther (Ls) was significantly smaller than short level pollen from the Mid morph (Ms). In *O. dregei* (population 59) pollen from mid and short level anthers of the Long morph did not differ in size (Lm = Ls). Both anthers of this morph produced smaller pollen grains than their equivalents in other morphs. In *O. nidulans* (population 103) anthers of all morphs produced pollen grains of similar size, showing no significant differences when adjacent levels were compared. In *O. purpurea* (population 47) pollen grains produced by anthers of the short level from Long morphs (Ls) were of the same size as those in the mid level anthers of the same morph (Lm) and Short morph (Sm), with no significant difference between them.

Although pollen from Short morph anthers (Sm and Sl) in *O. monophylla* L. (population 97) differed significantly in size, they did not correspond with pollen from same level anthers of other morphs. In its long anther (Sl), pollen grains were significantly smaller than pollen from

Table 4.1: Average pollen grain size and standard deviation (nm) for each anther level (long, mid, and short) in all morph types, Long (L), Mid (M), and Short (S), in 15 *Oxalis* populations. Population number (Pop n) and percentage morph representation (%L, %M, and %S) are also given. Different letters within a population indicate statistically significant differences based on Kruskal-Wallis multiple comparison z-value tests.

Species	Pop n	long anthers		mid anthers		short anthers		IE	% L	% M	% S	Kruskal-Wallis test
		M	S	L	S	L	M					
<i>O. adspersa</i>	6	50.5±9.5 a	47.7±3.1 a	40.4±2.9 b	42.1±4.0 b	30.3±2.7 c	35.0±3.8 d	*	33.0	21.7	45.3	H (5, N= 600) =473.62, $p=0.000$
<i>O. caprina</i>	10	-	34.8±5.4 a	-	33.4±4.9 a	-	-	**	0.8	0.0	99.2	H (1, N= 200) =2.63, $p=0.1048$
<i>O. cf. fergusoniae</i>	87	58.8±3.5 a	59.6±5.9 a	-	55.4±6.3 b	-	41.8±2.6 c	**	0.0	6.3	93.7	H (3, N= 400) =230.69, $p=0.000$
<i>O. dregei</i>	59	39.8±3.4 a	41.8±5.5 a	31.9±3.2 c	36.3±4.7 b	31.8±4.1 c	33.0±4.0 c	**	30.6	17.1	52.3	H (5, N= 600) =285.96, $p=0.000$
<i>O. hirta</i> var. <i>tubiflora</i>	15	71.8±5.6 a	72.0±3.6 a	51.5±4.6 c	62.7±2.8 b	46.6±2.3 d	49.8±2.6 c	ns	39.4	28.3	32.3	H (5, N= 600) =514.41, $p=0.000$
<i>O. hirta</i> var. <i>tubiflora</i>	21	69.1±4.6 a	69.5±5.1 a	60.5±3.9 b	59.2±4.1 b	49.4±3.1 c	48.7±2.6 c	ns	30.2	35.8	34.0	H (5, N= 600) =490.30, $p=0.000$
<i>O. hirta</i> var. <i>tubiflora</i>	28	72.5±3.7 a	68.6±3.8 a	60.1±5.3 b	59.3±2.9 b	50.2±5.3 c	52.8±3.4 c	*	33.3	44.4	22.2	H (5, N= 600) =471.11, $p=0.000$
<i>O. incarnata</i>	66	45.2±4.3 a	-	41.9±3.6 b	-	35.8±2.7 c	39.8±5.3 d	**	65.3	34.7	0.0	H (3, N= 400) =183.78, $p=0.000$
<i>O. incarnata</i>	90	-	-	40.7±4.1 a	-	34.2±2.8 b	-	**	100.0	0.0	0.0	H (1, N= 200) =97.47, $p=0.000$
<i>O. monophylla</i>	97	46.5±4.6 a	40.6±4.3 b	38.4±3.6 bc	36.4±2.9 c	30.3±3.2 d	31.4±3.3 d	ns	32.0	38.0	30.0	H (5, N= 600) =420.13, $p=0.000$
<i>O. nidulans</i>	98	36.6±2.1 a	-	-	-	-	31.6±3.0 b	**	0.0	3.0	97.0	H (1, N= 200) =105.13, $p=0.000$
<i>O. nidulans</i>	103	45.4±3.2 a	46.4±5.3 a	41.8±3.6 ab	41.3±3.9 ab	38.6±2.5 bc	37.5±1.8 bc	ns	35.2	36.2	28.6	H (5, N= 120) =59.57, $p=0.0000$
<i>O. purpurea</i>	47	56.5±3.4 a	57.2±3.1a	48.8±3.0 bd	47.1±2.7 bd	41.6±3.6 cd	38.5±2.4 c	**	1.0	6.9	92.2	H (5, N=440) =372.65, $p=0.000$
<i>O. recticaulis</i>	82	49.9±4.6 ab	51.9±3.3 a	47.8±4.4 bc	47.1±3.4 c	39.0±2.6 d	39.3±2.2 d	**	66.7	18.8	14.6	H (5, N= 600) =396.96, $p=0.000$
<i>O. sp.</i>	99	42.0±2.6 a	40.8±2.7 a	36.9±1.9 b	36.1±1.8 b	32.4±1.2 c	32.3±2.3 c	ns	34.4	30.0	35.6	H (5, N= 600) =477.42, $p=0.000$

Notes: IE - Significance determined through χ -square distributions with 2 degrees of freedom; $P<0.05$ (*) and $P<0.01$ (**) represent significant departures from isopleth, ns = not significant. Kruskal-Wallis test: H = number of ranks, N = total number of observations, z = multiple comparison z values, p = significance level (0.05) associated with z.

long anthers of the Mid morph (MI). The same was true for mid anther pollen (Sm), which was significantly smaller than pollen grains found in mid anthers of the Long morph (Lm). In *O. recticaulis* (population 82) pollen grains from long anthers of the Mid morph (MI) were intermediate in size between long anthers of the Short morph (SI) and mid anthers of the Long morph (Lm). *Oxalis caprina* L. (10) showed no significant difference in the size of pollen grains from the two sets of anthers of the Short morph (SI = Sm).

Discussion

Most South African *Oxalis* populations show clear uniformity of reproductive organ levels, conforming to normal patterns of tristylous expression (Weller, 1981; Weller, 1992; Weller *et al.*, 2007). Despite this, many sexual organ mismatches were identified, such as divergence in same level organs within different morphs and varying degrees of separation of organ levels. In other reported cases of tristylous species such alterations resulted in changed population morph frequencies (Barrett, 1992; Barrett *et al.*, 1997, Hodgins & Barrett, 2008). Among the South African *Oxalis* populations studied here, in contrast, the influence of organ arrangement on morph frequency was not clear. Some isoplectic populations had very low ratios of organ heights, while anisoplectic populations had high ratios of organ heights and high reciprocity indexes.

The effect of incomplete pollen trimorphism on unbalanced morph frequency in populations also did not present a clear relationship. Populations at isoplectic equilibrium expressed pollen trimorphism, but there were exceptions. Some anisoplectic populations lacked one or more morphs, prohibiting a full evaluation of pollen trimorphism expression. A better understanding of these interactions among South African members of *Oxalis* require further studies including more populations per species. The effectiveness of reciprocal herkogamy and its influence on morph ratios should also be further investigated.

Other characteristics exhibited by *Oxalis* species that deserve further investigation include flower colour and scent production. Corolla colour in some *Oxalis* species can vary to such an extent that different colour forms occur sympatrically. In at least one species, *O. cf. fergusoniae*, corolla colour correlates with morph type, which could have profound effects through differential pollinator attraction. Preliminary studies have further shown flower colour within *Oxalis* communities to be clustered (De Jager *et al.*, 2009). They are not structured by neutral processes or by phylogeny, but by pollinators. The aquatic species *O. dines* Ornduff, *O. disticha* Jacq., *O. dregei* and the only forest endemic, *O. incarnata*, are all distinctly fragrant, suggesting flower odour as possible pollinator attractant.

Collectively all of these morphological floral attributes will act on pollination success, and will thus affect the long-term expression of tristylly among South African members of *Oxalis* (Goldblatt & Manning, 2000; Graham & Barrett, 2004; Hodgins & Barrett, 2006). Sosenski *et al.* (2010) have shown that the breakdown of tristylly in *Oxalis alpina* (Rose) Knuth in the Sky Island region of the Sonoran Desert, is driven by phenotypic variation, with major changes in the morphology of Short and Long styled plants.

This is the first time that such parameters have been calculated and compared for so many South African *Oxalis* species and populations. The observed patterns of tristylly expression detected here are very interesting and the breeding system of *Oxalis* merit further study. This broad-scale overview study now needs to be followed up by a series of more detailed analyses of individual species and/or species complexes.

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1. Dissertation Structure
2. Tristyly in flowering plants: A review
3. Morph representation in <i>Oxalis</i> populations across the Cape Floristic Region
4. Tristyly and sexual organ morphology in South African <i>Oxalis</i>
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7. Tristyly and self- incompatibility in a polyploid South African <i>Oxalis</i> species
8. Tristyly in the genus <i>Oxalis</i> in South Africa: A review

Summary

Processes of tristily breakdown in American *Oxalis* have been related to loss of one stylar morph, particularly the mid morph. In contrast, South African *Oxalis* species were found to vary in terms of the extent and type of morph expression. Here we investigate four South African *Oxalis* species and unpack the patterns of morph expression and anisoplety distribution in terms of geographic location, sexual reproductive organ morphology, disturbance and vegetation type expressed in different populations. Patterns of tristily expression in South African *Oxalis* species are clearly different to those recorded among American members of the genus. South African anisopleptic populations varied in terms of the morphs that were minority or absent (not always the mid morph) and there was no clear relationship with geographic location.

Key words: Tristyly, species distribution, *Oxalis glabra*, *Oxalis nidulans*, *Oxalis pes-caprae*, *Oxalis purpurea*, Cape Floristic Region, South Africa.

Introduction

Heterostylous systems have been widely used as models to explore the function and adaptive significance of floral polymorphisms (Barrett *et al.*, 2000). The study of the adaptation of these systems is usually based on patterns of heterostyly expression that deviate from Darwin's (1877) first descriptions of tristily and distily. Tristily is a heterostylous system that includes three different floral morphs in a population and it is restricted to only six flowering plant families (Barrett, 2010). Preservation of the tristylous system is easily assessed through quantification of floral morphs due to frequency dependent selection in populations (Barrett, 1992).

Tristily breakdown in American *Oxalis* often entails the loss of one stilar morph (Weller, 1992). Although various selective forces have been found to result in tristily breakdown, modifications in morph frequencies, morph morphology, fitness or incompatibility reactions tend to occur mostly in the Mid morph (Ornduff, 1964, 1972; Weller, 1992). In *O. suksdorfii* Trel., which extends from southern British Columbia to central California, along the United States Pacific Coast, Mid morphs contribute limited pollen and few seeds towards sexual reproduction (Ornduff, 1964). Other species from section *Corniculatae* have similarly shown the reproductive capacity of Mid morphs to be different to that of Short and Long morphs. This may account for the disappearance of Mid morphs in populations and the adjustment in position of mid-level anthers in the other two morphs (Ornduff, 1972). In *O. alpina* (Rose) Knuth populations of southeastern Arizona, United States, Mid morphs have preserved their fertility, but Short and Long morphs have lost their incompatibility differentiation, which was hypothesized to favour these morphs during out-crossing (Weller, 1981, 1986). Furthermore, the degree of incompatibility modification in the Short and Long morphs was strongly associated with a reduced frequency of Mid morphs. Distylous populations lacking the Mid morph were further shown to be distributed in similar geographic regions (Weller *et al.*, 2007). The geographical

distribution of tristylous and distylous populations of *O. alpina* coincides with that of most *Oxalis* section *Ionoxalis* species in the United States and Mexico (Weller *et al.*, 2007).

South African *Oxalis* species that display signs of morphological deviation are not limited to specific areas, environments, or restricted to geographic areas, and we observed a great deal of variation in degree and direction of morph expression within any one of the study species at the population level (Chapter 3). Here we aim to compare the patterns of tristylous expression between South African and American *Oxalis* species in order to examine if there are similarities between the two. In particular, those few South African species for which more than three populations were studied (*O. glabra* Thunb., *O. nidulans* E. & Z., *O. pes-caprae* L. and *O. purpurea* L.) are compared to one another, and to *O. alpina* (following Weller *et al.*, 2007) in terms of their morph frequencies and geographic distribution.

Material and Methods

Information about all populations of each species was extracted from a previous study (Chapter 3). The four species analyzed were *O. glabra* (N = 6 populations), *O. nidulans*. (N = 5 populations), *O. pes-caprae* (N = 5 populations) and *O. purpurea* (N = 8 populations) (Table 5.1). Each species was analyzed individually, focusing especially on the anisoplectic populations. With the exception of two populations, population 3 (*O. glabra*) and population 70 (*O. purpurea*) that had close to 50 plants sampled due to their small population size, all other populations involved in the analysis had 100 plants sampled. Flowers were collected at least 2 meters apart to avoid the effects of clonality. Parameters considered included the most scarce morph, arrangement of sexual reproductive organs (organ height ratios), geographic location (major locality and GPS coordinates of each population), environmental disturbance and distribution of each species (Table 5.1). Five disturbance categories were demarcated, ranging from highly disturbed to pristine. Factors that influenced this ranking included exact locality

Table 5.1: Summary of *Oxalis* populations analyzed in terms of morph representation and reproductive organ heights. Major population localities, precise locality (latitude and longitude in decimal degrees) and site information including levels of disturbance and exposure is supplied. Long (L), Mid (M) and Short (S) morphs percentage and isoplethic equilibrium (IE) are given based on the total number of flowers sampled. Organ height ratios were obtained by dividing the average mid- and long-level organ heights by the average short-level organ height. Lloyd's reciprocity index (RI) values were calculated by dividing the average distance between adjacent levels by the average difference within levels (Lloyd *et al.*, 1990), with larger values indicating higher levels of reciprocity.

Identification	Pop code	Collector & Collection Number	Major locality	GPS coordinates	Vegetation type	Disturbance	Exposure	% L	% M	% S	IE	Organ height ratios	RI
<i>O. glabra</i>	3	S.S.Siqueira & J. Zietsman - 0001	Caledon	S 34 08.354' E 19 43.567'	Mountain Fynbos and Farmland	High	Shade/sun	0.0	83.9	16.1	**	1:1.1:1.7	na
<i>O. glabra</i>	30	S.S.Siqueira & J. Zietsman - 0055	Hermon	S 33 26.820' E 19 02.890'	Renosterveld	Pristine	Full sun	41.8	23.5	34.7	NS	1:1.5:2.3	6.0
<i>O. glabra</i>	46	S.S.Siqueira - 0003	Stellenbosch	S 33 55.964' E 18 52.563'	Fynbos	Disturbed	Full sun	38.9	30.5	30.5	NS	1:1.4:2.3	12.0
<i>O. glabra</i>	49	S.S. Turketti & S.S.Siqueira - 006	Atlantis	S 33 40.395' E 18 36.453'	Farmland	Very high	Partial shade	29.0	42.0	29.0	NS	1:1.4:2.3	25.3
<i>O. glabra</i>	52	S.S.Siqueira & C. Obone - 002	Ceres	S 33 25.371' E 19 15.902'	Farmland	Very high	Full sun	58.7	26.9	14.4	**	1:1.4:2.1	3.6
<i>O. glabra</i>	80	S.S. Siqueira, K. Oberlander & F. Roets - 0005	Stellenbosch	S 33 52.681' E 18 48.095'	Farmland	Very high	Full sun	29.5	39.7	30.8	NS	1:1.5:2.4	18.2
<i>O. nidulans</i>	31	L.L. Dreyer, J. Zietsman & F. Roets - 0001	Hermon	S 33 26.820' E 19 02.890'	Renosterveld	Pristine	Full sun	35.0	44.0	21.0	*	1:1.4:1.8	6.3

Identification	Pop code	Collector & Collection Number	Major locality	GPS coordinates	Vegetation type	Disturbance	Exposure	% L	% M	% S	IE	Organ height ratios	RI
<i>O. nidulans</i>	95	S.S.Siqueira & B. Marais - 0003	Bredasdorp	S 34 28.513' E 20 25.813'	Dune Fynbos	Fire	Full sun	35.4	32.3	32.3	NS	1:1.2:1.6	5.0
<i>O. nidulans</i>	98	L.L. Dreyer, K.J. Esler & S.S. Siqueira - 0001	Villiersdorp	S 33 57.761' E 19 30.575'	Renosterveld	Pristine	Shade	0.0	3.0	97.0	**	1:1.1:1.3	na
<i>O. nidulans</i>	101	L.L. Dreyer - 0608	Hermon	S 33 26.820' E 19 02.890'	Renosterveld	Pristine	Full sun	36.0	29.0	35.0	NS	1:1.5:2.4	8.4
<i>O. nidulans</i>	103	L.L. Dreyer - 0607	Hermon	S 33 26.820' E 19 02.890'	Renosterveld	Pristine	Full sun	35.2	36.2	28.6	NS	1:1.4:1.8	5.0
<i>O. pes-caprae</i>	7	S.S. Turketti & S.S.Siqueira - 0001	Villiersdorp	S 34 04.883' E 19 16.218'	Town surroundings & Renosterveld	Very high	Full sun	24.3	12.6	63.1	**	1:1.5:2.3	5.6
<i>O. pes-caprae</i>	29	S.S.Siqueira & J. Zietsman - 0054	Vanrhynsdorp	S 31 46.678' E 18 45.960'	Mountain Fynbos & Farmland	Low	Full sun	35.6	37.5	26.9	NS	1:1.4:2.3	13.5
<i>O. pes-caprae</i>	37	S.S.Siqueira & J. Zietsman - 0056	Nuwerus	S 31 14.952' E 18 17.946'	Succulent Karoo	Disturbed	Full sun	27.5	43.1	29.4	NS	1:1.4:2.1	40.7
<i>O. pes-caprae</i>	51	S.S.Siqueira & C. Obone - 0001	Ceres	S 33 29.564' E 19 11.710'	Farmland	Very high	Full sun	38.8	36.9	24.3	NS	1:1.4:2.2	7.3
<i>O. pes-caprae</i>	57	S.S.Siqueira & J. Zietsman - 0060	Touws River	S 33 12.295' E 19 43.812'	Tanqua Karoo	Pristine	Shade/sun	28.6	46.2	25.2	*	1:1.4:2.0	9.6
<i>O. purpurea</i>	11	J. Zietsman - 0011	Citrusdal	S 32 38.921' E 18 57.063'	Fynbos	High	Partial shade	32.4	41.2	26.5	NS	1:1.4:2.3	6.2
<i>O. purpurea</i>	25	S.S.Siqueira & J. Zietsman - 0030	Vanrhynsdorp	S 31 46.573' E 18 45.823'	Fynbos	Disturbed	Full sun	33.0	40.2	26.8	NS	1:1.4:2.3	5.6
<i>O. purpurea</i>	36	S.S.Siqueira & J. Zietsman - 0038	Nuwerus	S 31 14.392' E 18 32.146'	Succulent Karoo	Low	Full sun	37.9	29.1	33.0	NS	1:1.4:2.4	11.0

Identification	Pop code	Collector & Collection Number	Major locality	GPS coordinates	Vegetation type	Disturbance	Exposure	% L	% M	% S	IE	Organ height ratios	RI
<i>O. purpurea</i>	42	S.S.Siqueira & J. Zietsman - 0057	Clanwilliam	S 32 01.774' E 19 11.523'	Succulent Karoo	Disturbed	Full sun	34.3	37.1	28.6	NS	1:1.4:2.3	8.3
<i>O. purpurea</i>	43	S.S.Siqueira & J. Zietsman - 0058	Clanwilliam	S 32 01.774' E 19 11.523'	Succulent Karoo	Disturbed	Full sun	34.0	38.0	28.0	NS	1:1.4:2.3	6.2
<i>O. purpurea</i>	47	S.S.Siqueira & J. Zietsman - 0059	Malmesbury	S 33 26.573' E 18 36.592'	Farmland	Very high	Full sun	1.0	6.9	92.2	**	1:1.4:2.2	4.0
<i>O. purpurea</i>	56	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira - 0009	Calvinia	S 31 28.189' E 19 42.052'	Succulent Karoo	Disturbed	Full sun	38.6	25.7	35.6	NS	1:1.4:2.0	5.3
<i>O. purpurea</i>	70	S.S. Turketti & S.S.Siqueira - 0009	Villiersdorp	S 33 58.938' E 19 10.701'	Renosterveld	Disturbed	Full sun	36.5	40.4	23.1	NS	1:1.4:2.3	7.7

Notes: $P < 0.05$ (*), $P < 0.01$ (**), ns = not significant, as determined through G-statistics. Significance determined through χ -square distributions with 2 degrees of freedom, with cut off threshold of $G > 5.99$ and $G > 9.21$ respectively. Lloyd's reciprocity index: na = not applicable.

(edge of roads, farmland or natural field) and ecological aspects such as evidence of recent fires, vegetation type and vegetation structure. Information on the distribution of species was obtained from the Pretoria Computerized Information System (PRECIS) dataset, Salter (1944) and the *Oxalis* database at Stellenbosch University, South Africa. Distributions were inclusive of all varieties and forms recognized within each species.

Results

Species analyzed fell within two distribution categories. *O. nidulans* and *O. glabra* are range restricted with localized distributions within the Western Cape Province, while *O. purpurea* and *O. pes-caprae* are widespread, with ranges including two or more Provinces. *O. nidulans* has the narrowest range, occurring mainly in the Boland region of the southern Western Cape Province. It extends from Ceres and Tulbagh in the northwest to Cape Town in the south (Figure 5.1). *Oxalis glabra* has a wider range, occurring from the Overberg Region in the south to Clanwilliam in the northwest of the Western Cape Province. *O. purpurea* is widespread, occurring from Tsitsikama in the Eastern Cape Province, through Bredasdorp and the Overberg Region in the Western Cape Province, extending all the way north to Springbok in the Northern Cape Province. *O. pes-caprae* has the widest distribution. It occurs throughout most of the Eastern Cape Province, Western Cape Province and Northern Cape Province, and even extends into southern Namibia.

Of the five *Oxalis nidulans* populations analyzed, two were anisopletic, namely Hermon (population 31) and Villiersdorp (population 98). The sampling documented a new locality for this species from outside of the known range at Bredasdorp and this population was isopletic. The anisopletic population at Hermon had a reduced number of Short morphs, but all three morphs were present. It was located in a natural area with no obvious signs of disturbance. The organ height ratio and reciprocity index were both similar to the other two isopletic populations



Figure 5.1: Maps of four South African *Oxalis* species depicting their distribution range (shaded area) on the Western Cape Province of South Africa. A. *Oxalis nidulans*, B. *Oxalis glabra*, C. *Oxalis purpurea* and D. *Oxalis pes-caprae*. Symbols represent populations considered in this study; triangles are isopleptic populations, stars are anisopleptic populations.

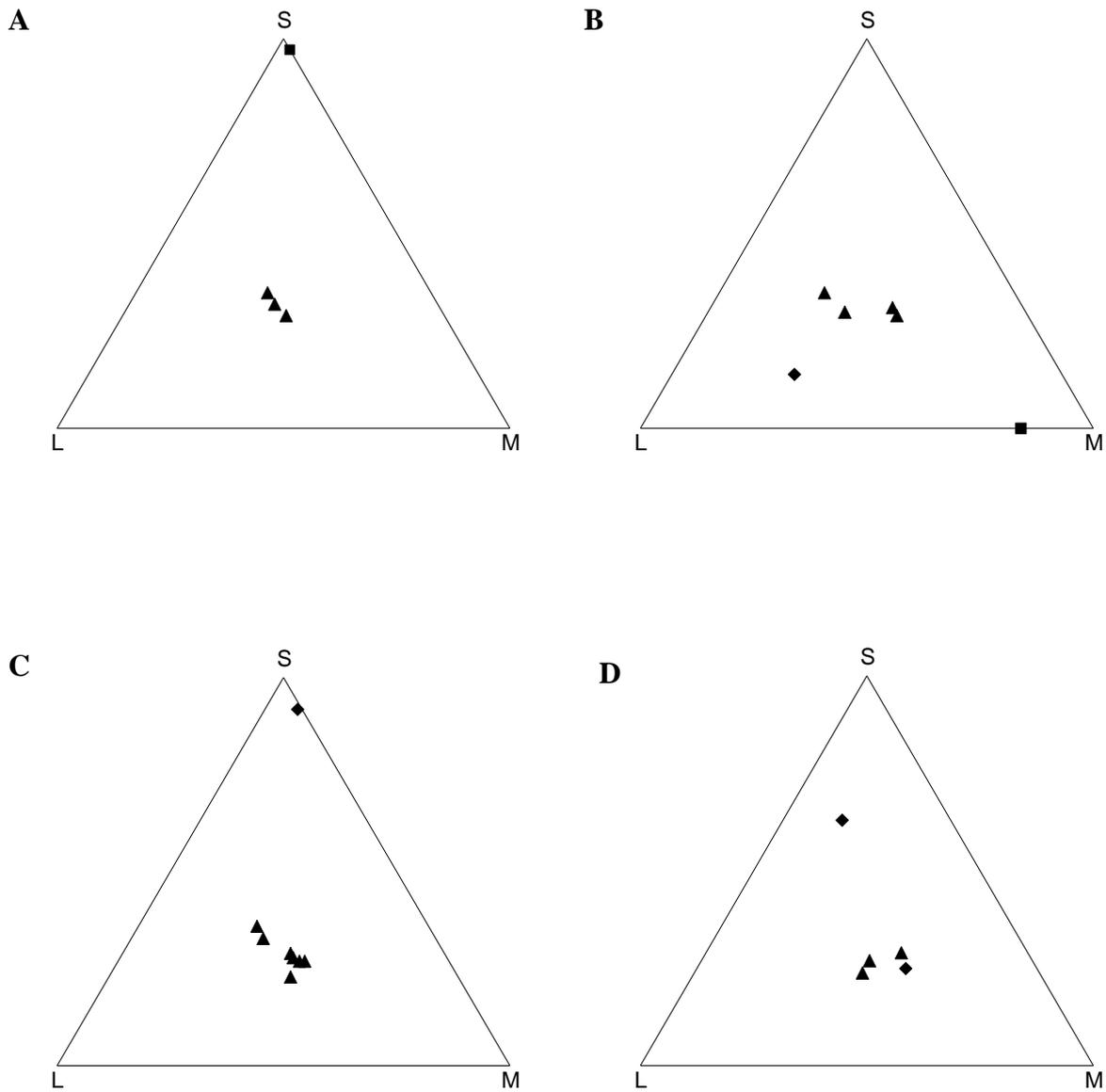


Figure 5.2: Ternary graphs representing floral morph frequencies in populations of four tristylous *Oxalis* species in South Africa. A. *Oxalis nidulans*, B. *Oxalis glabra*, C. *Oxalis purpurea* and D. *Oxalis pes-caprae*. Triangles represent trimorphic isoplectic populations, diamonds are trimorphic anisoplectic populations and squares are dimorphic populations. The cut off threshold for lack of isoplety was $G > 5.99$. Each symbol represents a single population and the distance of the symbol to a given tip is proportional to the frequency of that morph in that population. Isoplety is equidistant from all tips.

found in this same area. The anisopletic population at Villiersdorp comprised mainly of Short morphs (97%), with 3% Mid morphs and no Long morphs present at all (Figure 5.2 A). It was located in a natural area with low or no disturbance observed. Plants grew under the shade of other Fynbos plants. The organ height ratio of this population was the lowest of all *O. nidulans* populations studied (1:1.1:1.3), while the lack of Long morphs made it impossible to calculate the reciprocity index.

The six *Oxalis glabra* populations analyzed occurred in the centre of the distribution range of this species. Two populations were anisopletic (population 3 and 52), while the other four were isopletic. The two anisopletic populations occurred at similar latitudes towards the western side of the range of this species, but one was much further North than the other. These anisopletic populations varied in terms of the morph that dominated, with the Ceres population (population 52) including 59% Long morphs, while the Caledon population (population 3) included 84% Mid morphs. All three morphs were present in the Ceres population, while the Caledon population entirely lacked the Short morph (Figure 5.2B). Both populations occurred in highly disturbed areas, but this was also the case for most other *O. glabra* populations studied. The reciprocity index of the Ceres population was the lowest of all *O. glabra* populations studied (3.6), while the lack of Short morphs made this value impossible to calculate in the Caledon population. The latter population, however, had the lowest organ height ratio (1:1.1:1.7) of all *O. glabra* populations.

O. purpurea populations were sampled from a wide area, including eight populations of which only the Malmesbury population (population 47), located in the middle of the range of this species, was anisopletic. This population included 92% Short morphs, with only 7% Mid morphs and 1% Long morphs (Figure 5.2 C). Although the ratio of organ heights was comparable to that of isopletic populations, the reciprocity index of this anisopletic population

was very low (4.0) compared to the average reciprocity index for the other populations of this species (7.2). Most populations of this species occurred in fairly disturbed areas.

Five populations of *O. pes-caprae* were studied, of which two were anisopletic (population 7 and 57). The anisopletic populations occurred very far from each other. At Villiersdorp (population 7), Short morphs dominated by 63%, while at Touws River (population 57) Mid morphs dominated by 46%. Both populations included all three morphs (Figure 5.2D). Population 7 had the lowest reciprocity index (5.6) in *O. pes-caprae* and was growing in a very disturbed area. Population 57 had a higher reciprocity index (9.6) and was growing in an area with low disturbance. Interestingly, two of the isopletic populations were also growing in very disturbed areas (population 37 and 51).

Discussion

The distribution of tristylous and distylous populations of *Oxalis alpina* in the Sonoran Desert follow a geographic pattern with distylous populations concentrated in northeast Arizona and tristylous populations towards eastern and southeastern Arizona, New Mexico and Sonora (Mexico), associated with different degrees of incompatibility in the Short and Long morphs (Weller *et al*, 2007). Furthermore, there is a consistent pattern in the morph affected, with all distylous populations losing the Mid morph. No such pattern emerged for the South African *Oxalis* species studied. In the four species analyzed, any one of the three morphs could be present in reduced frequencies in the anisopletic populations. Moreover, anisopletic populations of South African *Oxalis* species did not follow a geographic pattern comparable to that of *O. alpina*. They were not concentrated in any particular geographic area, and followed no distinct gradient. However, patterns did suggest possible factors that could be driving lack of morphological equilibrium.

The reproductive organ arrangement of the anisoplectic *Oxalis nidulans* population at Villiersdorp was drastically changed compared to the other populations of this species; 7.9% of the flowers analyzed had reproductive organ arrangement as Short morphs, 3.0% had arrangement as Mid morphs and in 89.1% had a row of anthers at the upper level, but stigmas and the mid set of anthers were located at the same level. Although this arrangement was found only in this population, this could be the cause of its unbalanced morph frequency. Distylous *Oxalis alpina* populations were composed of Short and Long morphs, also presenting an alteration in organ height, but different from *O. nidulans* in having the Long morph producing short and mid level anthers at the same level (mid). This morphological arrangement in *O. alpina* was combined with a deviation in the incompatibility reactions of these two morphs; illegitimate inter-morph crosses with mid level anthers fertilized both long and short stigmas (Weller *et al.*, 2007). The incompatibility reaction between morphs in *O. nidulans* at Villiersdorp was also found to deviate as self and intra-morph crosses produced seeds. This is explored in the next chapter, together with the reproductive organ arrangement in species of section *Sagittatae*, which resembles three-dimensional reciprocity (Armbruster *et al.*, 2006). In contrast, the anisoplectic population at Hermon presented no differences in reproductive organ arrangement, organs height ratios or reciprocity index. In fact, this population was located in the same environment as two other isoplectic *O. nidulans* populations growing under no or limited levels of disturbance in a nature reserve. This population should be further investigated to identify possible factors influencing tristylous morph equilibrium maintenance.

Oxalis purpurea populations mainly grew in disturbed areas surrounded by different vegetation types. The anisoplectic population of this species differs from the isoplectic populations in having a lower reciprocity value and no natural vegetation in their surrounding areas. Most of the western and northwestern side of this species' distribution range was sampled, while sampling from the more eastern side of the range was scant. Given the extended distribution range of this

species, no finite conclusions should be drawn before more populations are studied. *O. purpurea* produces flowers of different colours in different parts of its range, which should also be taken in consideration when analyzing the breeding system of this species (De Jager *et al.*, 2009; Zietsman, 2007).

Anisopletic populations of *O. glabra* presented different patterns of tristily expression. They differed in terms of the predominant morph, the surrounding vegetation and the levels of disturbance. Population 3 at Caledon had a very low organ height ratio with Mid morph stigmas and anthers of the lower level at the same height (mid), as in semi-homostylous flowers. In contrast the other anisopletic population, population 52, has flowers with organs arranged normally, without obvious alterations in organ levels. Both populations are located close to the Great Escarpment of the Western Cape Province. The escarpment mountains run parallel to the coast, resulting in a series of east-west trending mountain ranges in the southern half of the Cape Region and north-south trending ranges in the west (Goldblatt & Manning, 2000; Linder, 2003). *Oxalis* species diversity in this Province follows this geographical gradient. The majority of taxa are located at the widest area of the narrow coastal plain that includes the Cape Peninsula and the west and south coasts. *Oxalis* species decrease in number towards the mountains, where elevations abruptly reach an average of 1000m (Oberlander *et al.*, 2002). Perhaps tristily expression in *O. glabra* follow the same pattern as *Oxalis* species distribution, with anisoplety setting in at higher altitudes near the escarpment. This must be verified through the addition of populations sampled throughout the range of this species.

Tristily expression in *O. pes-caprae* presented no apparent relationship with any of the parameters analyzed. Most populations were growing in very disturbed areas and the surrounding vegetation varied greatly. The reciprocity index was lower in only one of the anisopletic populations and neither of the anisopletic populations presented alterations of organ

heights. The distribution range of this species in South Africa exceeds that of most *Oxalis* species, and in order to obtain a clear picture on the causes of deviations on tristylous expression in this species additional populations and parameters should be examined.

The five parameters used for comparison of tristylous expression in this study provided adequate comparison to differentiate patterns among South African *Oxalis* species from those among American members of the genus. Although the spectrum of this study did not allow us to draw major conclusions, results clearly direct future approaches to the exploration of tristylous breakdown in South African *Oxalis*.

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1. Dissertation Structure
2. Tristyly in flowering plants: A review
3. Morph representation in <i>Oxalis</i> populations across the Cape Floristic Region
4. Tristyly and sexual organ morphology in South African <i>Oxalis</i>
5. Patterns related to morph frequency in tristylous <i>Oxalis</i> species
6. Three-dimensional reciprocity in <i>Oxalis</i>
7. Tristyly and self- incompatibility in a polyploid South African <i>Oxalis</i> species
8. Tristyly in the genus <i>Oxalis</i> in South Africa: A review

Summary

Section *Sagittatae* is distinguished from other South African members of *Oxalis* (Oxalidaceae) by a unique suite of floral characters. Here we investigate the floral morphology of members of section *Sagittatae* and compare them to characters of *Linum suffruticosum* from which a variation of distyly was described. The flowers of two representative species from this section were analyzed and compared in seven populations. This was followed by a more detailed analysis of corolla and reproductive organ morphology, organ level segregation and arrangement and incompatibility expression in three of these populations. In members of section *Sagittatae* sexual reproductive organs are arranged by height, angle of reflex from the central floral axis and by the orientation of anthers and stigmas. This is similar to the arrangement in *L. suffruticosum*, and therefore represents a tristylous form of three-dimensional reciprocity. The systems in members of section *Sagittatae* and *L. suffruticosum* are similar, but not identical. The lack of self-incompatibility in three-dimensional tristily described here renders it more reliant on reciprocity. Successful cross-fertilization appears to be highly dependent on pollinator efficiency for pollen transfer.

Key words: three-dimensional tristily, heterostyly, self-compatibility, *Oxalis*, *O. eckloniana*, *O. nidulans*, South Africa.

Introduction

There are six classes of stilar polymorphisms reported from flowering plants: heterostyly including distyly and tristyly, stigma-height dimorphism, enantiostyly, flexistyly and inversostyly (Barrett, 2010). Flexistyly and inversostyly have been described fairly recently and recorded in isolated cases, while all other stilar polymorphisms have a long study history and have been reported in many families globally (Darwin, 1877; Li *et al.*, 2001; Pauw, 2005; Barrett, 2010). All of these stilar polymorphisms are identified by the position of their sexual reproductive organs within a flower, their stilar and anther lengths and/or the segregation of their organs in time (flexistyly).

Despite the amazing variety and spatial arrangements of sexual reproductive organs, all these polymorphisms are believed to have the same outcome, the promotion of cross-fertilization (Armbruster *et al.*, 2006; Barrett, 2002; Li *et al.*, 2001; Pauw, 2005). The floral designs assist the exchange of genetic material and maximize parental fitness, thereby reducing inbreeding depression. Darwin (1877) described heterostyly as a system that facilitates insect-mediated pollination, reducing pollen wastage (disassortative pollen flow). Most workers agree with this interpretation of heterostyly as an outbreeding mechanism (Hernández & Ornelas, 2007; Hodgins & Barrett, 2008; Wolfe & Barrett, 1989). The recently described polymorphisms flexistyly and inversostyly are regarded as more efficient strategies of pollen dispersal seeing that through morphology they enhance the precision of pollen placement on the insect's body (Armbruster *et al.*, 2006; Li *et al.*, 2001; Pauw, 2005).

South Africa includes 201 *Oxalis* species, all of which are generally considered to be tristylous (Salter, 1944; Dreyer *et al.*, 2009). Tristylous flowers include two sets of anthers and one set of stigmas that alternate reciprocally at three different levels in three different morphs, Long, Mid and Short. The South African section *Sagittatae* was delimited based on the shared presence of a

unique suite of floral morphological characters (Salter, 1944). Salter (1944) described the reproductive structures of members of this section as follows: “When released from the tube of the corolla, the two lower sets (whether stamens or styles) spread out widely and only the longest set stands erect”. The anthers of this section are sagittate in shape, contrasting to the oval anthers of all other South African *Oxalis* species. In other South African sections all three levels of sexual reproductive organs stand erect as a column in the center of the flower.

Armbruster *et al.* (2006) found a variation of distyly in *Linum suffruticosum* L. with bent styles and stamens, achieving a three-dimensional arrangement. This resembles the arrangement of floral organs displayed in *Oxalis* Section *Sagittatae*. Here we assess the arrangement of sexual reproductive organs in two representative species of section *Sagittatae* and compare it to the arrangement in *L. suffruticosum*. The incompatibility reactions in members of section *Sagittatae* were also tested to assess its expression in these morphologically different *Oxalis* species.

Material and Methods

Salter (1944) included the following five species in section *Sagittatae*: *Oxalis eckloniana* Presl, *O. nidulans* E. & Z., *O. minuta* Thunb., *O. fibrosa* Bolus f. and *O. microdonta* T.M.Salter. They are all centred in the southwestern part of the Cape Floristic Region (CFR) of South Africa. *O. fibrosa*, *O. minuta*, and *O. microdonta* are very range restricted and rare, while *O. eckloniana* and *O. nidulans* are more widespread and abundant. In this study we focused mainly on the two widespread species, *O. eckloniana* and *O. nidulans*.

Floral morph frequencies were calculated in two populations of *O. eckloniana* and five populations of *O. nidulans*. For this 100 plants were sampled at least 2 meters apart from each other to avoid the effects of clonality. Results were compared for goodness-of-fit to an isoplethic (1:1:1) equilibrium using G-statistics (Sokal & Rohlf, 1995). Following methods proposed by

Lloyd *et al.* (1990), organ separation and the reciprocity index were calculated for each population. Stigma and anther lengths were measured in 20 flowers per morph in each of these seven populations. The ratio of the organ heights was calculated by dividing the average of middle and upper level organ heights by the average of lower level organ heights calculated collectively. This measured the relative separation of the three organ levels. The reciprocity index was calculated by dividing the average distance between adjacent levels by the average distance within levels. This measured the uniformity of the segregation between organ levels in each population. Larger values indicate higher levels of reciprocity and *vice-versa*. Digital photographs (Canon PowerShot A530 – Canon South Africa (Pty) Ltd, Halfway House; and Nikon SMZ 645 – Nikon South Africa, Kyalami Business Park, Midrand) were taken of the reproductive organ arrangement in fresh flowers.

Based on their morph representation, three populations were selected to assess corolla morphology, reproductive organ morphology and the extent of self-incompatibility expression. Populations in which morphs deviate from equilibrium (anisoplectic populations) often also display modifications in the arrangement of sexual reproductive organs and/or self-incompatibility, both of which potentially impact fertilization success (Weller, 1992). We chose one isoplectic population of *O. eckloniana* (Gydo population) with an intermediate ratio of organ heights, and two populations of *O. nidulans*, one isoplectic population at Elandsberg Nature Reserve (Elandsberg 1) with a high ratio of organ heights, and one anisoplectic population at Jonaskop with the lowest organ height ratio of all *O. nidulans* populations studied.

Fresh flowers (n = 10) of each morph from all of these three populations were collected and analysed. The following measurements were taken: flower diameter, petal laminae length, floral tube length, floral tube width at apex, upper reproductive level length, middle reproductive

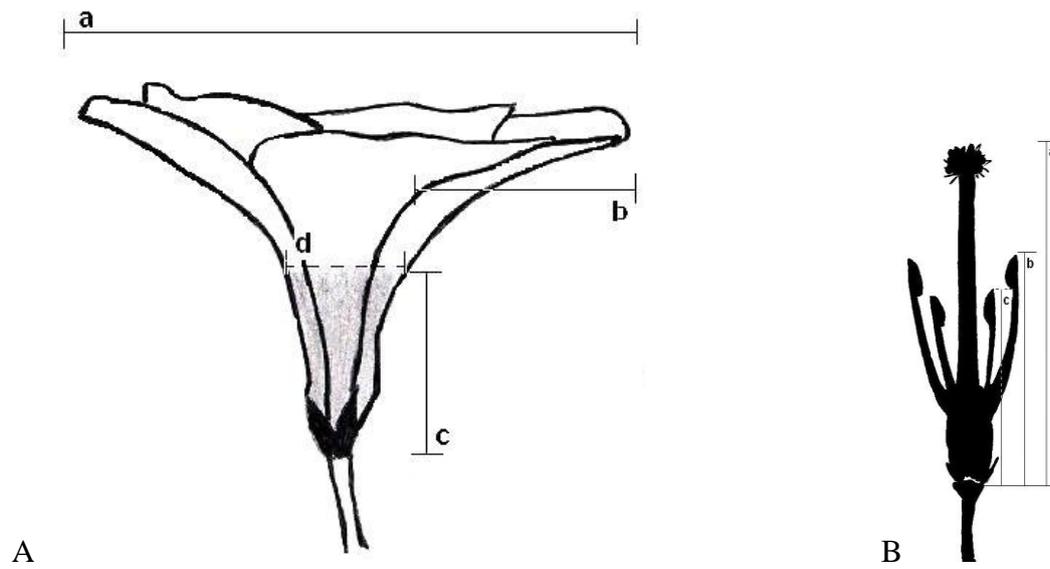


Figure 6.1: Diagrammatic representation of the floral measurements taken for flowers of each morph of each of the three populations studied. A: a. flower diameter, b. petal laminae length, c. floral tube length, d. floral tube width at apex, B: reproductive organ heights at a. upper level, b. middle level, c. lower level.

level length, lower reproductive level length (Figure 6.1). Sexual reproductive organs were measured from the base of the ovary to the highest point of a given level using electronic callipers (TA – Toolquip, CE, 0-150 mm). Pollen grains of each anther level of each morph were measured with a Nikon Eclipse E400 light microscope (40x magnification) fitted with a micrometer. Descriptive statistics (Statistica 8, StatSoft Southern Africa – Research (Pty) Ltd., Johannesburg, South Africa) were applied to all data, and results are given as means with standard deviation of values.

Self-incompatibility was tested through legitimate, open and self-pollination experiments in the field during the 2008 flowering season. Legitimate pollinations were carried out in emasculated flowers on the first day of anthesis. Legitimate pollen collected from anthers of other morphs was brushed onto stigmas. For self-pollinations, pollen from the two anther whorls was brushed onto the stigma of the same flower of each morph type. Hand-pollinated flowers were bagged and tagged to avoid insect interference. Open-pollinated flowers were tagged and left open to insect visitation. In *O. nidulans* at Jonaskop, intra-morph pollinations were performed instead of legitimate pollinations due to the lack of compatible anthers. The very low frequency of Mid morphs present in this population limited the number of pollinations that could be performed. Seed set was compared between the different crosses and results were analyzed through Kruskal-Wallis ANOVA by ranks (Statistica 8, StatSoft Southern Africa – Research (Pty) Ltd., Johannesburg, South Africa).

The population selection further enabled us to investigate the relationship between reproductive organ morphology and self-incompatibility. We assumed these two aspects of tristylly expression to be expressed independently, following Lloyd and Webb (1992).

Results

Six of the seven populations studied were at isoplethic equilibrium, while the Jonaskop population of *O. nidulans* deviated significantly from equilibrium (Table 6.1, $P < 0.01$, $G = 317.17$). Long morphs were absent from this population, 3% of individuals were of the Mid morph, while 97% of individuals were of the Short morph.

The organization and structure of sexual reproductive organs in flowers of *Sagittatae* species differed from all other southern African *Oxalis* species. In the usual arrangement upper, middle and lower level organs are all erect in the center of the flower tube (Fig. 6.2 a). They maintain this position even after the removal of the corolla. In section *Sagittatae* flowers of the upper level organs are erect and in the center of the flower tube, but the middle and lower level organs spread outwards, pressing anthers and/or stigmas against the inside of the corolla tube (Fig. 6.2 b and 6.3). When the corolla is removed, the Mid and Short morph styles spread even further outwards (Fig 6.4 a-f).

The orientation of anthers and stigmas are also unique in members of section *Sagittatae*. Usually anthers and stigmas of the upper level face upwards towards the opening of the floral tube, while middle and lower level anthers and stigmas are turned outwards to face the inside of the corolla tube (Fig. 6.2 a). In section *Sagittatae*, only stigmas at the upper level of Long morphs face upwards as in other southern African *Oxalis* species (Fig. 6.5 a-c). Upper level anthers in Mid and Short morphs face each other (orientated inwards). Anthers and stigmas of middle and lower levels all face inwards towards the center of the floral tube. The anthers of all morphs at all levels are sagittate in shape, instead of oval as in all other South African species (Fig 6.6).

The analysis of organ heights revealed that populations of the same species present similar separation of levels, although a few outliers were detected (Table 6.1, ratio of average heights).

Table 6.1: Morph frequency, ratio of organ heights and reciprocity indices of *Oxalis eckloniana* and *O. nidulans* populations. Percentages of morph frequencies (Long, Mid, and Short) are based on the number of flowers analyzed (N) per population. *G* is the likelihood ratio between sampled and expected frequencies. Ratios of organ heights were obtained by dividing the average middle and upper level organ lengths by the average lower level organ lengths. The reciprocity index was obtained by dividing the average distance between adjacent levels by the average distance within levels.

Species and locality	Morph frequency (%)			N	G	Ratio of organ heights	Reciprocity Index
	Long	Mid	Short				
<i>O. eckloniana</i> populations							
Caledon District – R316	31.5	35.2	33.3	108	0.22 ns	1:1.3:1.7	5.4
Ceres District - Gydopas	27.9	35.7	36.4	129	1.77 ns	1:1.3:1.6	6.0
<i>O. nidulans</i> populations							
Clanwilliam District	31.6	37.8	30.6	98	0.86 ns	1:1.3:1.5	9.1
Bredasdorp District - De Hoop	35.4	32.3	32.3	99	0.18 ns	1:1.2:1.6	5.0
Villiersdorp District - Jonaskop	0.0	3.0	97.0	165	317.73**	1:1.1:1.3	na
Hermon District - Elandsberg 1	36.0	29.0	35.0	100	0.88 ns	1:1.5:2.4	8.4
Hermon District - Elandsberg 2	35.2	36.2	28.6	105	1.1 ns	1:1.4:1.8	5.0

** $P < 0.01$ significance determined from χ -square distribution (G statistics), ns = not-significant; ratio of organ heights: S:M:L; reciprocity index: na = not applicable.

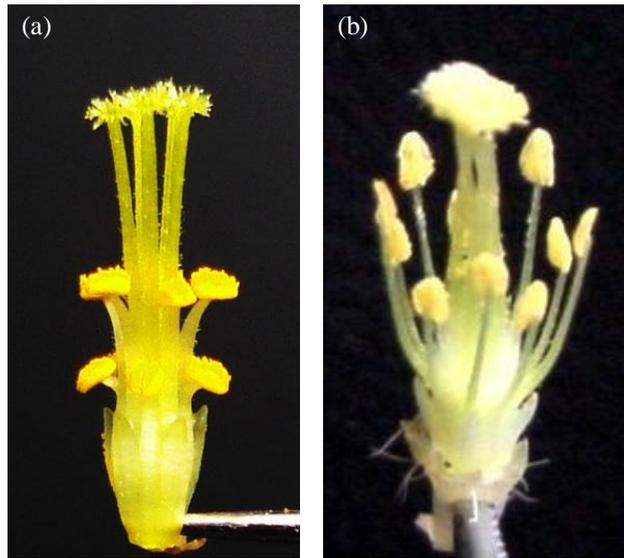


Figure 6.2: Reproductive organ arrangements in flowers of South African *Oxalis* species: (a) usual organ arrangement in a Long morph flower (*O. pes-caprae*), (b) organ arrangement in a Long morph flower in species in section *Sagittatae* (*O. nidulans*).

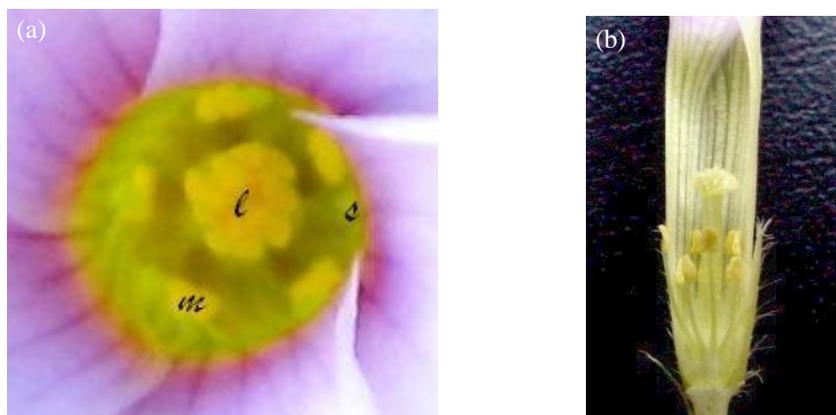


Figure 6.3: Reproductive organ arrangements in flowers of species in section *Sagittatae*. (a) *O. nidulans* - Short morph - view of flower from above, showing the inside of the corolla tube (*l* - upper level anthers, *m* - middle level anthers, *s* - lower level stigmas), (b) *O. eckloniana* - Long morph - side view showing middle and lower level anthers pressed against the inside of the floral tube.

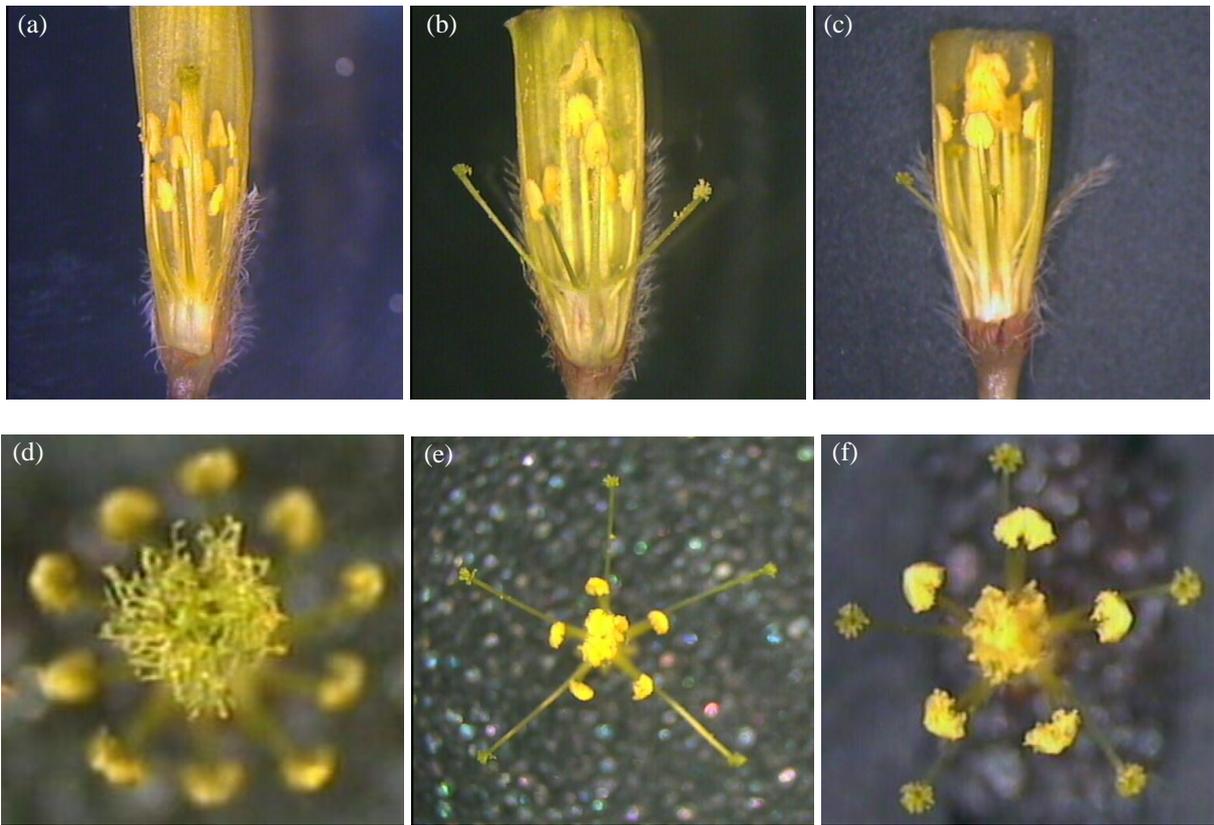


Figure 6.4: *O. nidulans* - a-c: Side view of reproductive organ arrangement within the corolla tube in the three morphs. (a) Long morph, (b) Mid morph, (c) Short morph. d-e: Sexual reproductive organs arrangement in the three morphs seen from above after corolla removal. (d) Long morph, (e) Mid morph, (f) Short morph.



Figure 6.5: *O. nidulans* - Orientation of stigmas and anthers. (a) Upwards orientation of stigmas of Long morph flowers (x25 magnification), (b) Inwards orientation of stigmas in Mid and Short morph flowers (x50 magnification), (c) Attachment and inwards orientation of middle and lower level anthers (x50 magnification).

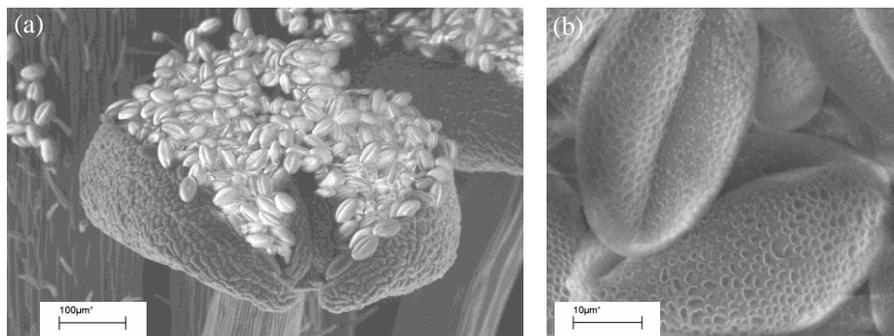


Figure 6.6: Electron micrographs of anthers and pollen grains of *O. nidulans*. (a) SEM micrograph showing the shape of the anther at dehiscence (b) individual pollen grains.

The Jonaskop population of *O. nidulans* had the smallest ratio of organ heights (Table 6.1, 1:1.13:1.31), while the Elandsberg population had the highest ratio of organ heights (Table 6.1, 1:1.47:2.37). Smaller values indicate that the sexual reproductive organs are closer together, while higher values indicate greater separation between reproductive organ levels. The reciprocity index could not be calculated for the Jonaskop population due to the absence of Long morphs and the very low percentage of Mid morphs in this population. The remaining six populations all had reciprocity indices that conform to typical tristylous species (Lloyd & Webb, 1992; Barrett, 1993).

Anthers within a given morph showed significant differences in pollen grain size between the two anther whorls, while same level anthers from different morphs had pollen of the same size (Table 6.2). Larger pollen grains were found in upper level anthers, middle level anthers had intermediate sized pollen grains and lower level anthers had the smallest pollen grains.

Corolla measurements were very constant in terms of laminae and tube length between the different populations of the two species (Table 6.2). In all populations of both species the upper organ level was always shorter than the corolla tube, and thus never protruded from the tube (Table 6.2).

From the crosses performed for the incompatibility assessment both legitimate and illegitimate (self and intra-morph) pollination resulted in seed production in all populations (Table 6.3). The self-incompatibility expression was found to be markedly different between the two *O. nidulans* populations. In the Jonaskop population, most seeds resulted from self-pollination ($n = 265$), with seeds also produced by intra-morph pollination ($n = 79$), a pollination type that usually produces no seeds. There were no statistical differences in seed production resulting from self- and intra- morph pollination in this population. Open pollination yielded very surprising results

Table 6.2: Pollen grain sizes (nm) and corolla dimensions (mm) of *O. nidulans* and *O. eckloniana* populations according to morph type (Long, Mid and Short). Pollen grain sizes are given per anthers (long, mid and short). Corolla dimensions include laminae width and length, tube width and length and reproductive organ heights (upper, middle and lower). Data are presented as mean \pm standard deviation.

Populations	Morph type	Pollen sizes			Laminae		Tube		Levels		
		long	mid	short	width	length	width	length	upper	middle	lower
<i>O. eckloniana</i> populations											
Ceres District - Gydopas	Long		40.1 \pm 2.4	38.1 \pm 2.9	20.32 \pm 2.43	9.36 \pm 1.50	3.59 \pm 0.21	13.00 \pm 1.02	9.94 \pm 0.62	7.62 \pm 0.62	6.02 \pm 0.45
	Mid	47.4 \pm 2.4		37.0 \pm 1.3	21.40 \pm 2.41	9.36 \pm 1.32	3.67 \pm 0.38	13.75 \pm 1.05	9.27 \pm 0.53	7.62 \pm 0.70	5.98 \pm 0.38
	Short	46.6 \pm 3.0	40.5 \pm 2.1		22.26 \pm 2.95	9.94 \pm 1.08	3.39 \pm 0.41	13.75 \pm 1.51	9.39 \pm 0.45	7.54 \pm 0.28	5.34 \pm 0.45
<i>O. nidulans</i> populations											
Villiersdorp District - Jonaskop	Long		-	-	-	-	-	-	-	-	-
	Mid	-		-	-	-	-	-	8.37 \pm 0.40	7.49 \pm 0.43	6.52 \pm 0.51
	Short	36.6 \pm 2.1	-	31.6 \pm 3.0	22.65	11.65	3.15	11.36	8.72 \pm 0.59	7.26 \pm 0.35	6.55 \pm 0.73
Hermon District - Elandsberg 1	Long		41.8 \pm 3.6	38.6 \pm 2.5	17.33 \pm 0.28	9.73 \pm 1.55	3.03 \pm 0.28	11.69 \pm 1.86	9.13 \pm 0.40	7.07 \pm 0.48	5.37 \pm 0.28
	Mid	45.4 \pm 3.2		37.5 \pm 1.8	16.46 \pm 1.08	9.3 \pm 1.39	3.05 \pm 0.44	11.33 \pm 0.87	8.91 \pm 0.51	6.81 \pm 0.24	5.39 \pm 0.20
	Short	46.4 \pm 5.3	41.3 \pm 3.9		17.54 \pm 1.22	9.61 \pm 1.47	3.08 \pm 0.25	11.80 \pm 1.13	8.72 \pm 0.50	7.00 \pm 0.48	5.00 \pm 0.48

Table 6.3: Seed production in *O. nidulans* and *O. eckloniana* populations, according to pollination type (legitimate, open, self, and intra-morph). Data are presented as the mean \pm standard deviation (of the total number of seeds produced (ns) and the total number of crosses (ncr). Kruskal-Wallis ANOVA provide statistical comparisons between the different types of pollinations for each population

Species	Location	legitimate		open		self		intra		Kruskal-Wallis ANOVA
		mean \pm SD (ns)	ncr	mean \pm SD (ns)	ncr	mean \pm SD (ns)	ncr	mean \pm SD (ns)	ncr	
<i>O. nidulans</i>	Jonaskop	-	-	0 \pm 0 (0)	12	12.05 \pm 13.7 (265)	22	11.29 \pm 14.7 (79)	7	H (2, N= 41) =9.112339 p =.0105
<i>O. nidulans</i>	Elandsberg	11.55 \pm 5.3 (338)	29	7.65 \pm 6.2 (222)	29	6.06 \pm 6.3 (182)	30	-	-	H (2, N= 88) =10.90897 p =.0043
<i>O. eckloniana</i>	Gydo	17.52 \pm 8.9 (508)	29	2.07 \pm 5.4 (54)	26	2.33 \pm 5.4 (49)	21	-	-	H (2, N= 76) =42.16418 p =.0000

Kruskal-Wallis test: H = number of ranks, N = total number of observations, z = multiple comparison z values, p = significance level (0.05) associated with z.

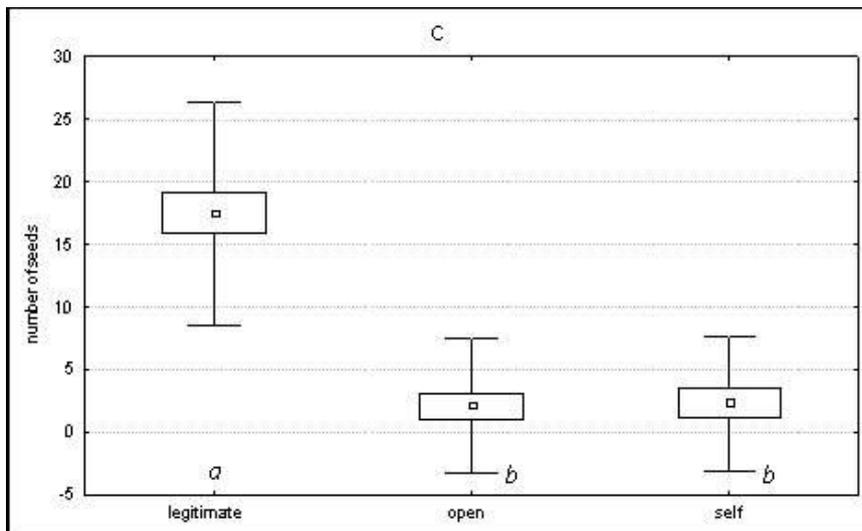
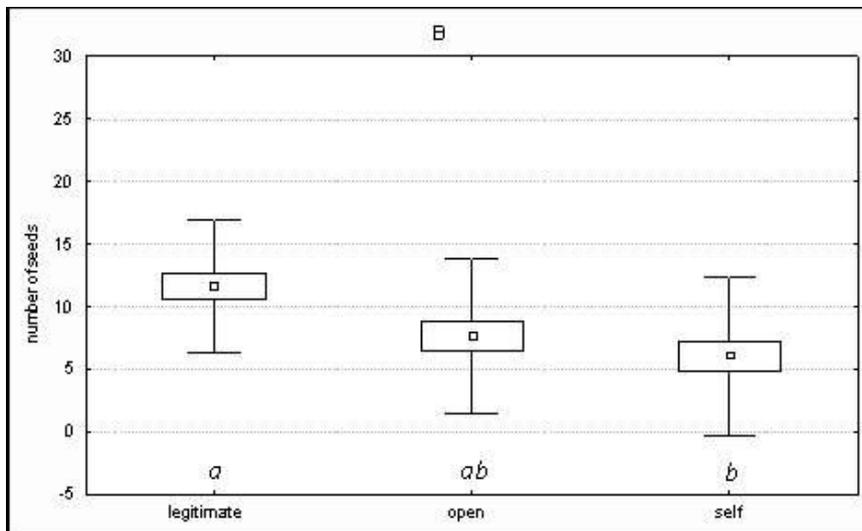
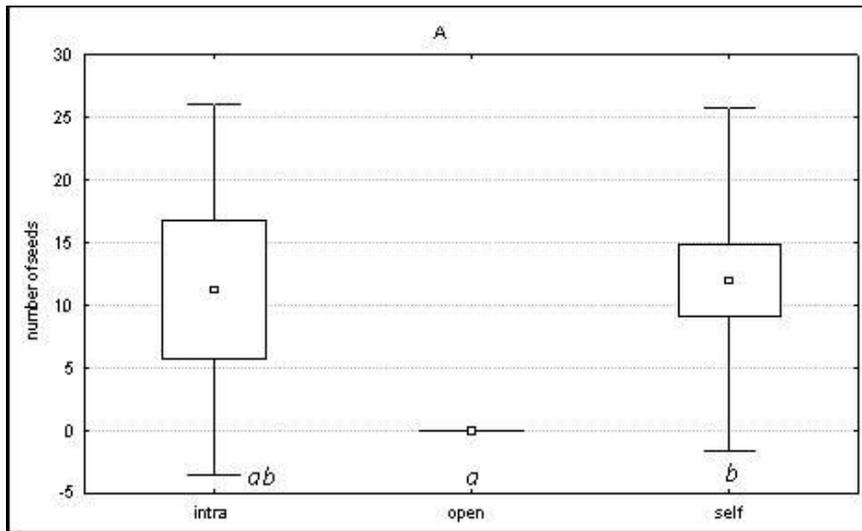


Figure 6.7: Box & Whisker plots representing seed production per population. A. *Oxalis nidulans* – Jonaskop, B. *O. nidulans* – Elandsberg, C. *O. eckloniana* – Gydo. Legitimate, open and self pollination experiments were performed in the field during the 2008 flowering season to test incompatibility. In *O. nidulans* at Jonaskop, intra-morph pollinations were performed instead of legitimate pollinations due to lack of compatible anthers. Different small letters (*italics* type) represent statistical differences (Kruskal-Wallis ANOVA). Y-axis: number of seeds, X-axis: crosses by category, small squares: average, large rectangle: standard error, vertical lines: standard deviation.

in that no seeds were formed (Figure 6.7 A, $z = 1.60$, H: 2, N= 41). The Elandsberg population of *O. nidulans* yielded different crossing experiment results. Legitimate pollination resulted in the most seeds ($n = 338$), while open pollination resulted in fewer seeds ($n = 222$). There were no significant differences in seed production between legitimate and open pollination or self pollination and open pollination (Figure 6.7 B, $z = 1.07$, H: 2, N =88). Self pollinations yielded the least seeds ($n = 182$), but seed production was still high compared to open pollinations.

Crossing experiment results of *O. eckloniana* were similar to results of the Elandsberg population of *O. nidulans*. Legitimate pollinations produced the most seeds, and here they differed significantly from both self and open pollinations ($n = 508$ vs. 54, Figure 6.7 C, $z = 5.02$; $n = 508$ vs. 49, $z = 5.55$). Self and open pollinations did not differ significantly in numbers of seeds produced ($n = 49$ and 59 respectively, $z = 0.80$).

Discussion

Three-dimensional reciprocity was recently described in *Linum suffruticosum* as a variation of heterostyly where anthers and stigmas show reciprocity in three dimensions instead of in only one (height) (Armbruster *et al.*, 2006). *L. suffruticosum* is a dimorphic species with populations comprised of two morphs, thrum and pin. Important aspects of this system include intermorph differences in angles of divergence of styles and stamens from the central axis of the flower and the degree of rotation of the styles and filaments. This orientation results in dorsal (nototribic) pollen placement by short-styled (thrum) flowers and ventral (sternotribic) pollen placement by long-styled (pin) flowers, as stigmas of thrums contact the ventral side of the pollinator and the stigmas of pins contact the dorsal side.

The arrangement of reproductive structures in tristylous *Oxalis* species in section *Sagittatae* is similar to the arrangement in distylous *L. suffruticosum*. In all three morphs in *Oxalis*, filaments

and/or styles at the middle and lower levels flex away from the central floral axis, pressing anthers and stigmas against the inside of the corolla tube. These flowers thus acquire a three dimensional arrangement, with reproductive parts separated both by organ height and the distance of the organs from the central axis of the flower. In addition, anthers and stigmas are twisted and orientated in different ways at different heights, adding yet another element to the arrangement of sexual reproductive organs in these flowers. This floral structure corresponds exactly to the three-dimensional arrangement described in *L. suffruticosum*, and therefore represents the first example of this strategy within a tristylous species.

Armbruster *et al.* (2006) confirmed that the *Linum* system is truly distylous, because it is associated with differences in style and stamen length, pollen size and morphology, equilibrium of morphs in populations and intramorph incompatibility. The assessment of the breeding system of section *Sagittatae* species revealed aspects that still suggest the presence of fully functional tristylous. Style and stamen lengths remain very constant in height such that upper, middle and lower level organs match between morphs, indicating reciprocity between levels. Pollen grain sizes also decrease from long to short level stamens, and this remains constant between the morphs. In contrast, the high levels of self-compatibility suggest breakdown of the tristylous system among members of this section.

The Jonaskop population of *O. nidulans* has a 1:1.1:1.3 ratio of organ heights suggesting failure in reciprocity, as organs of different heights are borne in very close proximity. Furthermore, this population lacks self-incompatibility in both the Mid and the Short morphs. Finally this population is anisoplectic; it completely lacks the Long morph, has a very low percentage of Mid morphs and is dominated by the Short morph

Self-incompatibility was, in fact, absent from all three *Sagittatae* populations studied. Self-pollinations resulted in seed set both in the Jonaskop and Elandsberg 1 populations of *O. nidulans* and in the *O. eckloniana* population at Gydo. Intra-morph pollinations at Jonaskop also resulted in seed production. As argued by Armbruster *et al.* (2006), equal morph ratios can result from reciprocity of sexual reproductive organs and effective disassortative pollen transfer, even when self-incompatibility is not functional or expressed. This is the case in the Elandsberg 1 population of *O. nidulans* and the Gydo population of *O. eckloniana* in which the three morphs were in equilibrium despite the lack in self-incompatibility. The anisoplety at the Jonaskop population of *O. nidulans* is thus not necessarily the result of self-compatibility, but may be ascribed to the lack of reciprocity of its sexual reproductive organs not generating disassortative pollination. This needs confirmation through future detailed analyses of all incompatibility reactions of that and other populations of section *Sagittatae* species.

Despite obvious similarities between the distylous *Linum* and the tristylous *Oxalis* systems, there are also significant differences between the two. In the *Linum* system spatial separation of reproductive structures occurs at two levels and involves two morphs, while the *Oxalis* system separates sexual reproductive organs at three levels and involves three morphs. Distyly is still fully functional in the *Linum* system, with self-incompatibility still fully expressed, reciprocity of organs and all populations at isopleletic equilibrium. In the *Oxalis* system there are signs of breeding system breakdown and weak reciprocity of morphs present in at least one anisopleletic population. Preliminary experiments suggested that reproductive organ arrangement increased intermorph pollen flow between rather than within morphs in the *Linum* system (Armbruster *et al.*, 2006). The *Oxalis* system indicates the same efficiency, because although morphs were self-compatible, populations were isopleletic, suggesting that the reciprocity of organs guaranteed their equilibrium.

Fertilization success in flowers displaying reproductive polymorphisms depends upon both pollinator behaviour and flower morphology (Pauw, 2005; Sun *et al.*, 2007). In *Pontederia cordata* L., the pattern of insect visitation determines the rate of fertilization success, where only the combination of insect fauna guarantees pollen from all morphs and levels to be successfully transferred (Wolfe & Barrett, 1989). In all recently described polymorphisms, out-crossing success depended either on the positioning of the insect when approaching the flower (inversostyly), the visitation pattern of pollinators (flexistyly), or the size of the insect proportional to the flower (inversostyly, flexistyly and three-dimensional reciprocity) (Armbruster *et al.*, 2006; Sun *et al.*, 2007). This dependency on pollinator type and behaviour in tristylous three-dimensional reciprocal system in species of section *Sagittatae* should be tested. Tristylous three-dimensional reciprocity, as expressed in members of section *Sagittatae*, is unique in terms of stilar polymorphisms and will allow us to enhance the understanding of the impact of pollinator diversity and behaviour on the functionality of stilar polymorphisms.

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1. Dissertation Structure
2. Tristyly in flowering plants: A review
3. Morph representation in <i>Oxalis</i> populations across the Cape Floristic Region
4. Tristyly and sexual organ morphology in South African <i>Oxalis</i>
5. Patterns related to morph frequency in tristylous <i>Oxalis</i> species
6. Three-dimensional reciprocity in <i>Oxalis</i>
7. Tristyly and self- incompatibility in a polyploid South African <i>Oxalis</i> species
8. Tristyly in the genus <i>Oxalis</i> in South Africa: A review

CHAPTER 7 - TRISTYLY AND SELF INCOMPATIBILITY IN A POLYPLOID SOUTH AFRICAN *Oxalis*
SPECIES

Summary

Heterostyly is expressed at many levels that usually combine morphological and physiological aspects. When a heterostylous species also presents polyploidy these aspects have shown to vary through loss of polymorphisms and/or gain of self-compatibility. *Oxalis hirta* var. *tubiflora* is a tristylous polyploid taxon native to South Africa. Here we analyse the expression of self-incompatibility in three populations of this variety through a complete pollination experiment. Results are then compared to the morphological, genetic and physiological attributes of this taxon. Seed production in *Oxalis hirta* var. *tubiflora* followed the expected pattern of tristylous species, with legitimate pollinations resulting in the most seeds and illegitimate pollinations producing no or very few seeds. Flower morphological variation was observed in one population only.

Key words: Tristyly, self-incompatibility, polyploidy, triploid, hexaploid, fertility, pleiocotyly, *Oxalis hirta*, South Africa.

Introduction

Heterostyly is the combination of floral polymorphism and herkogamy (Darwin, 1877). It has been recognized as a system that promotes out-crossing, avoiding self-pollination through morphological arrangement of the sexual reproductive organs (for a review see Barrett, 1992, 2002). This system contains complex morphological components, while physiological self-incompatibility occurs simultaneously in many cases (Dulberger, 1992). Distyly and tristyly are heterostylous systems and they differ mainly in the number of morphs present per population. Distylous populations include only two morphs (Pin and Thrum), while tristylous populations include three morphs (Long, Mid and Short). In species with typical trimorphic incompatibility, two different incompatibility reactions are present within a single morph (self- and inter-morph pollination), with only six cross combination being compatible and considered legitimate pollinations that should result in seed formation (Darwin, 1877; Weller, 1992). Many families, in which the morphological aspects of tristyly are still intact, show breakdown in self-incompatibility expression (Barrett *et al.*, 1997; Eckert & Mavraganis, 1996; Glover & Barrett, 1983). Among American *Oxalis* species, the extent of self-incompatibility expression was shown to vary considerably (Guth & Weller, 1986; Marco & Arroyo, 1998; Mulcahy, 1964; Ornduff, 1964, 1983; Trognitz *et al.*, 2000; Weller, 1976, 1979, 1981; Weller & Denton, 1976), while the few South African *Oxalis* species studied also showed deviations in self-incompatibility expression (Zietsman, 2007).

Salter (1944) recognized *Oxalis hirta* L. as a group species that includes the following varieties: *O. hirta* var. *hirta* (including 6 forms); var. *canescens* Knuth; var. *tenuicaulis* Knuth; var. *intermedia* Salter; var. *polioeides* Salter; var. *tubiflora* (Jacq.) Salter and var. *secunda* (Jacq.) Salter. The varieties are defined based on both vegetative and floral morphological characters, with *Oxalis hirta* var. *tubiflora* representing the most distinctive variety by far (Dreyer & Johnson, 2000). Unlike in flowers of the majority of *Oxalis* species, the tubes are more than

three times as long as the laminae of the petals, and extremely narrow (mostly 20 – 44 mm long and 2 – 4.5 mm wide). The tube morphology is reminiscent of long-proboscid fly pollinated flowers of the guild described by Goldblatt and Manning (1999) and Manning and Goldblatt (1996), and long proboscid flies were indeed observed visiting these flowers both in natural populations and in the Botanical Garden at Stellenbosch University (pers. obs.).

O. hirta var. *tubiflora* occurs in a very restricted area of the Western Cape Province, South Africa, extending from Citrusdal, through Clanwilliam to Vanrhynsdorp. The furthest populations occur only a 170 km apart (Figure 7.1). One population of this taxon was found to deviate significantly from isoplethy (Chapter 3). Dreyer (1996) described the pollen of this taxon as supra-areolate, a very unique pollen type restricted to the well-supported monophyletic *O. hirta* clade in South Africa (Oberlander *et al.*, 2004; Oberlander *et al.*, 2009). Dreyer and Van Wyk (1998) described extensive aberrant pollen grain formation within this taxon, while Suda (pers. comm.) found populations of this taxon to be either triploid or hexaploid, with a basic chromosome number of $x = 7$.

Polyploidy has caused no seed production in *Oxalis* species growing outside of their native range (eg. *O. pes-caprae* L.), therefore relying exclusively on clonal propagation (Castro *et al.*, 2007; Luo *et al.*, 2006). In addition, polyploid heterostylous species have been reported to have higher rates of self-fertilization and being related to homostyly (Barringer, 2007; Guggisberg *et al.*, 2006, but see Shore *et al.*, 2006). The present study assesses potential associations between ploidy level and trait expression in *O. hirta* var. *tubiflora*. In addition it tests the self-incompatibility expression of *O. hirta* var. *tubiflora* and evaluates the expression thereof against the background of its unique biological characters. Given its restricted geographical range, it was further feasible to assess populations from across the entire distribution area.

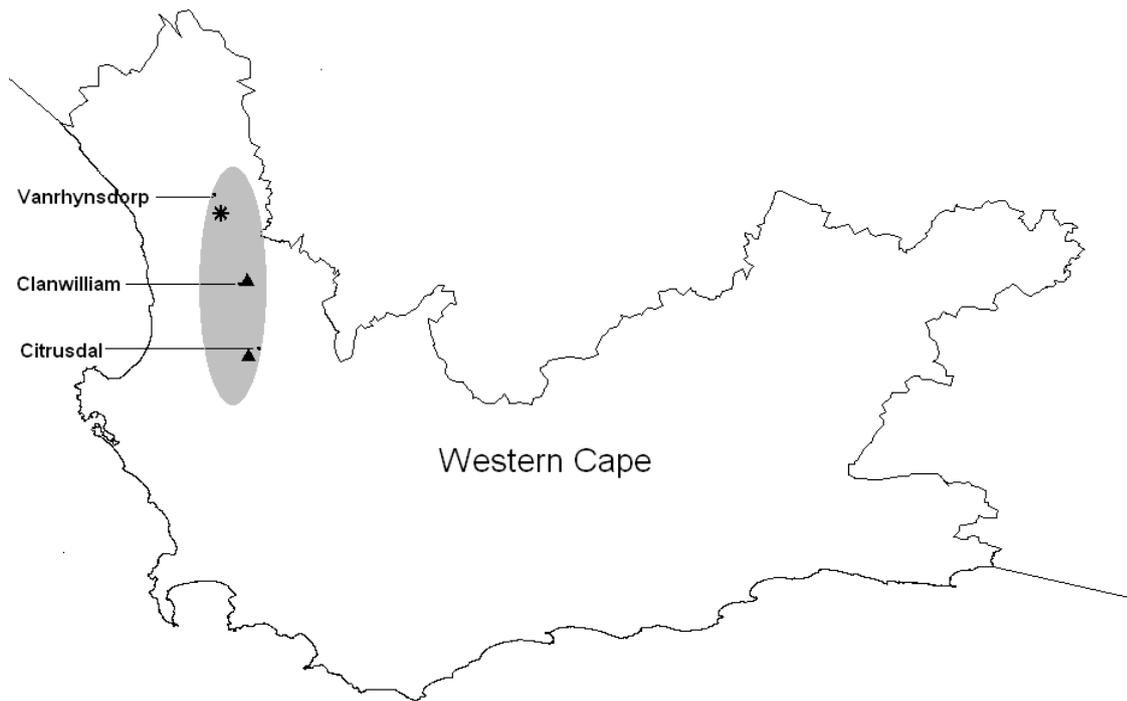


Figure 7.1: Natural distribution of *Oxalis hirta* var. *tubiflora* in the Western Cape Province of South Africa (shaded area). Individual symbols represent sampled populations for the self-incompatibility assessment. Triangles are populations at isopletic equilibrium, while the star indicates the anisopletic population.

Material and Methods

Three populations of *Oxalis hirta* var. *tubiflora* were selected to test self-incompatibility expression, two of which were isoplectic (Citrusdal and Clanwilliam populations), while the third population from Vanrhynsdorp was anisoplectic. Fifty bulbs were collected per population in 2005, including at least 15 bulbs of each morph type. They were potted up and cultivated in the Stellenbosch University Botanical Gardens, South Africa. During the flowering seasons of 2006, 2007 and 2008 all 24 possible pollination types were manually performed. These included illegitimate self-, intra-, and inter-morph pollinations, and legitimate pollinations. At least 10 repetitions were performed per cross type per morph.

Individual flowers were selected on the first day of anthesis, and their corollas and all their anthers were carefully removed. These anthers were used to perform all the different combinations of artificial crosses. After a flower was hand-pollinated it was bagged using light weight paper bags. Pollinated flowers were monitored for fruit and seed production. Seed set was compared between the different crosses and results were analyzed using Kruskal-Wallis ANOVA by ranks (Statistica 8, StatSoft Southern Africa – Research (Pty) Ltd., Johannesburg, South Africa). The viability of all seeds produced, irrespective of the cross combination from which it resulted, was tested through germination experiments. The seeds were collected from mature capsules and placed on a layer of moist filter paper in petri-dishes under ambient laboratory conditions. The petri-dishes were studied under a stereo-microscope (Nikon 300 - Nikon South Africa, Kyalami Business Park, Midrand) daily for 30 days, and seedling development was monitored and recorded.

Floral morphological characters such as reproductive organ level heights, reciprocity indices and pollen morphology of these populations were extracted from Chapter 4 and interpreted along with results of the artificial crosses. The ploidy level of the three populations was provided by

Results

The Clanwilliam and Vanrhynsdorp populations presented complete pollen trimorphism; pollen from long level anthers of Mid and Short morphs corresponded in size, as did pollen from mid level anthers of Long and Short morphs and pollen from short level anthers of Long and Mid morphs (Figure 7.2). Incomplete pollen trimorphism was found only in the Citrusdal population where neither pollen grains from mid level anthers or pollen from short level anthers corresponded in size. Instead pollen from mid level anthers of Long morphs corresponded in size to pollen from short level anthers of Mid morphs. These pollen grains from mid level anthers of Long morph were smaller than grains from mid level anthers of Short morphs, but larger than pollen from short level anthers of Long morphs. Figure 7.2 illustrates organ heights of all three morphs in all three populations studied. The maximum value along the Y axis equals the average length of the flower tube (40 mm). There were no significant differences between organ heights within same levels in a population (Kruskal-Wallis ANOVA by ranks). The Vanrhynsdorp population had flowers with shorter sexual reproductive organs compared to the other two populations. Ratio of organ heights was constant among populations, as were reciprocity index values (Table 7.1). Suda (pers. comm.) found the Citrusdal and Clanwilliam populations to be triploid (3x), while the Vanrhynsdorp population was reported to be hexaploid (6x) (Table 7.1).

Seeds produced by the different types of crosses in *Oxalis hirta* var. *tubiflora* followed the expected outcome in species that have tristylous self-incompatibility; legitimate crosses (stigmas receiving pollen from anthers of the same height) produced the most seeds, while illegitimate crosses (inter-morph, intra-morph and self pollinations) produced very few seeds (Table 7.2).

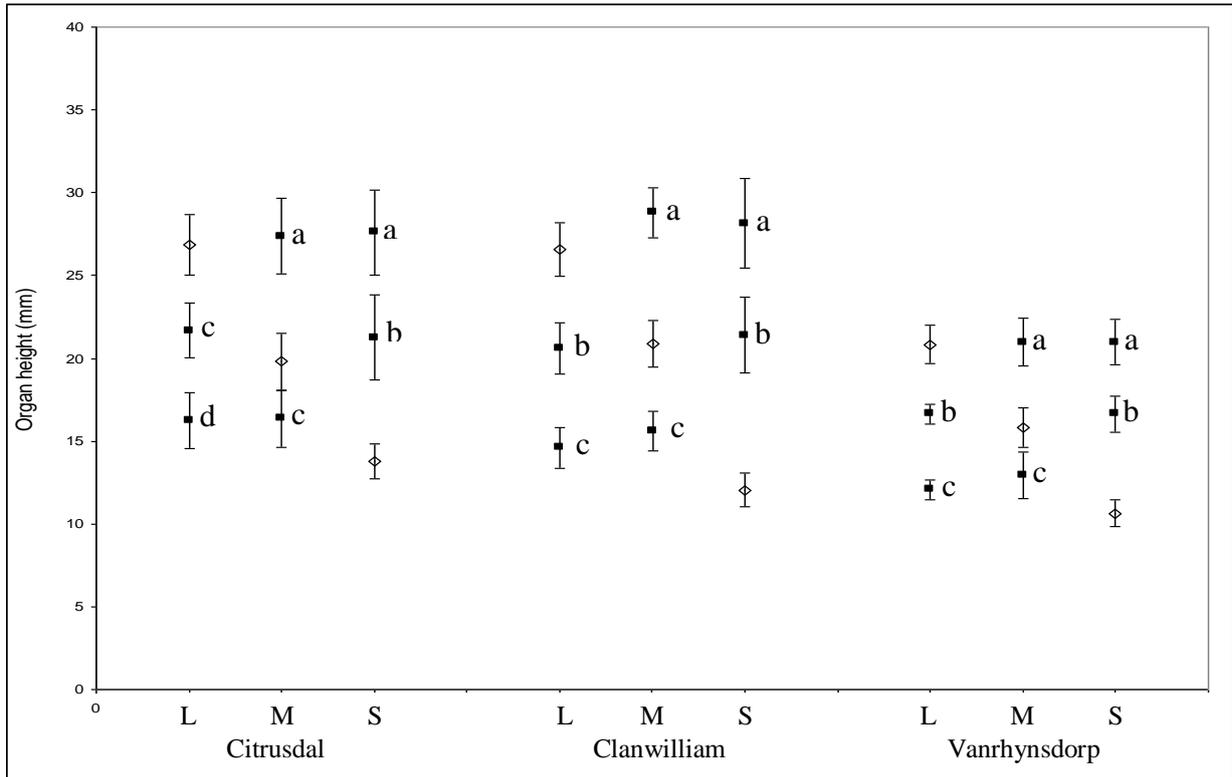


Figure 7.2: Graphical representation of reproductive organ heights of Long (L), Mid (M) and Short (S) morphs in the three populations studied. Solid blocks represent the average anther heights, while open diamonds represent average stigma heights and vertical bars depict standard deviation of these values. Different letters within the same population represent statistically significant differences in pollen grain sizes.

Table 7.1: Populations selected for inclusion in the crossing experiments, along with locality data (GPS co-ordinates in decimal degrees), morph representation (percentage of Long : Mid : Short morphs), G values and reported ploidy level. Organs height ratios were obtained by dividing the average mid- and long-level organ heights by the average short-level organ heights. Lloyd's reciprocity index (RI) values were calculated by dividing the average distance between adjacent levels by the average difference within levels (Lloyd *et al.*, 1990). Larger values indicate higher reciprocity.

Species	Collector - Collection Number	Locality	GPS co-ordinates	Morph representation			G value	Ploidy level	Organs height ratio	RI
				% L	% M	% S				
<i>O. hirta</i> var. <i>tubiflora</i>	S.S.Siqueira & J. Zietsman - 0017	Citrusdal	S 32 37.786° E 18 56.797°	39.4	28.3	32.3	1.86 ns	3X	1:1.4:1.8	5.1
<i>O. hirta</i> var. <i>tubiflora</i>	S.S.Siqueira & J. Zietsman - 0025	Clanwilliam	S 32 08.885° E 18 56.504°	30.2	35.8	34.0	0.53 ns	3X	1:1.5:2.0	4.7
<i>O. hirta</i> var. <i>tubiflora</i>	S.S.Siqueira & J. Zietsman - 0033	Vanrhynsdorp	S 31 43.859° E 18 46.432°	33.3	44.4	22.2	6.8 *	6X	1:1.4:1.8	6.3

Significance determined through χ -square distributions with 2 degrees of freedom. $P < 0.05$ (*) represent significant departure from isopleth, as determined through G-statistics. ns = not significant.

Table 7.2: Seed production in *O. hirta* var. *tubiflora* populations following different pollination types (legitimate, inter-morph, intra-morph and selfing). Data are presented as the mean \pm standard deviation of the total number of seeds produced (ns – bold type face) and the total number of crosses (ncr). Different small letters in *italics* represent statistical significant differences in seed production within populations according to the Kruskal-Wallis test.

Species	Locality	Morphs	Legitimate		Inter-morph		Intra-morph		Selfing	
			mean \pm SD	ns (ncr)	mean \pm SD	ns (ncr)	mean \pm SD	ns (ncr)	mean \pm SD	ns (ncr)
<i>O. hirta</i> var. <i>tubiflora</i>	Citrusdal	Long	2.7 \pm 2.3 <i>a</i>	56 (21)	0.1 \pm 0.3 <i>b</i>	1 (15)	zero <i>b</i>	0 (15)	zero <i>b</i>	0 (17)
		Mid	2.5 \pm 2.1 <i>a</i>	58 (23)	0 \pm 0.2 <i>b</i>	1 (20)	zero <i>b</i>	0 (17)	0.7 \pm 1.5 <i>ab</i>	11 (15)
		Short	2.1 \pm 2.0 <i>ab</i>	39 (19)	0 \pm 0.2 <i>b</i>	1 (20)	zero <i>b</i>	0 (16)	zero <i>b</i>	0 (15)
<i>O. hirta</i> var. <i>tubiflora</i>	Clanwilliam	Long	2.7 \pm 2.1 <i>ab</i>	49 (18)	0.3 \pm 0.7 <i>a</i>	3 (10)	zero <i>ac</i>	0 (2)	zero <i>ac</i>	0 (4)
		Mid	2.9 \pm 2.3 <i>a</i>	55 (19)	0.1 \pm 0.3 <i>bc</i>	2 (17)	zero <i>c</i>	0 (19)	zero <i>c</i>	0 (19)
		Short	3.4 \pm 2.4 <i>a</i>	76 (22)	0.1 \pm 0.7 <i>c</i>	3 (18)	zero <i>c</i>	0 (24)	zero <i>c</i>	0 (26)
<i>O. hirta</i> var. <i>tubiflora</i>	Vanrhynsdorp	Long	2.1 \pm 1.4 <i>ab</i>	46 (22)	0.2 \pm 0.4 <i>c</i>	3 (16)	0.2 \pm 0.6 <i>bc</i>	2 (10)	0.1 \pm 0.3 <i>c</i>	1 (10)
		Mid	2.5 \pm 1.3 <i>a</i>	51 (20)	0.3 \pm 0.9 <i>c</i>	5 (16)	0.3 \pm 0.8 <i>bc</i>	5 (15)	0.3 \pm 1.2 <i>c</i>	5 (17)
		Short	2.6 \pm 1.5 <i>a</i>	59 (23)	0.5 \pm 1.3 <i>c</i>	9 (18)	zero <i>c</i>	0 (23)	0.1 \pm 0.5 <i>c</i>	3 (23)

Short morphs at the Citrusdal population produced less seeds following legitimate crosses than the other two morphs. In this population self-pollination of the Mid morph also led to some seed set (in three of the 15 crosses), resulting in 11 seeds in total. In the Clanwilliam population Short morphs produced the most seeds after legitimate crosses, and all inter-morph pollination had at least one cross repetition that produced seeds. No intra-morph or self pollinations resulted in seed production. In the Vanrhynsdorp population seeds were formed following all cross combinations, although legitimate crosses always produced the most seeds. At least one repetition of each cross combination produced seeds. All seeds produced from all cross combinations from all populations were viable. They all germinated (100% germination) within 48 hours and developed into viable seedlings that survived for at least 30 days.

Discussion

Ploidy is common among *Oxalis* species (Dreyer & Johnson, 2000; de Azkue, 2000). In polyploid species seed production may be compromised by imbalances during meiosis, faulty gametophyte formation, gametophyte infertility or ovule abortion (Castro *et al.*, 2007; Luo *et al.*, 2006). Although *O. hirta* var. *tubiflora* has been reported to form abnormal pollen grains (Dreyer & Van Wyk, 1998) and to include different levels of ploidy (Suda, pers. com.) this did not affect fertility in any of the morphs in the three populations studied here. They all proved fully capable of producing seeds following legitimate pollinations.

Furthermore, polyploid heterostylous species have been reported to share two attributes: (1) they have, on average, higher rates of self-fertilization than their diploid relatives, and (2) polyploidy is often correlated with homostyly (Barringer, 2007; Guggisberg *et al.*, 2006, but see Shore *et al.*, 2006). *Oxalis hirta* var. *tubiflora* clearly presented strong incompatibility reactions after self and intra-morph pollinations, expressing very low levels of self-compatibility that varied from

population to population. Inter-morph crosses resulted in seed production in all three populations, but at very low frequencies (only 0.2 seeds per cross).

The Citrusdal population showed the strongest expression of self-incompatibility. However, Mid morphs at Citrusdal produced seeds after self pollination, suggesting that incompatibility reactions could have been altered in stigmas of this morph. The relaxation of incompatibility in this morph may be related to altered pollen incompatibility due to variations in pollen grain dimensions, as was reported in *Linum* (Dulberger, 1992). Indeed, morphological variations were observed in Mid morphs of this population in terms of mid stigmas arrangement, which were below the mid level anthers, and pollen size from lower level anthers, which were equivalent in size to pollen from mid level anthers of the Long morph. An indirect indication of change in pollen fecundity was the lower seed production after legitimate crosses in Short morphs compared to the other two morphs in this population. If pollen grains from lower level anthers in the Mid morph experience altered incompatibility, this should affect fertilization of short stigmas as well, reducing the success of fertilization in Short morphs by half as it would then exclusively rely on pollen from lower level anthers of the Long morph. The variation in mid stigma receptivity and pollen incompatibility behaviour in Mid morphs at Citrusdal should be tested further before any final conclusions can be drawn. The reported triploid state of this population is very curious, and strongly contradicts the results of high seed set found in this population. Triploid species are sterile because they do not have the required homologous pairs of chromosomes for successful gamete formation during meiosis and their chromosomes cannot divide evenly during cell division. They are mostly reported to be sterile, but varying levels of fertility in triploids have been reported (Soltis *et al.* 2004). Verification of the ploidy level and detailed micro- and macro-sporogenesis analyses will be required to understand how seed set is possible in these two populations.

The Vanrhynsdorp population was the most self-compatible, producing seeds following all cross types. *Oxalis tuberosa* Mol., an octoploid species from the high Andes, formed seeds after illegitimate crosses, but at equally high levels as seed set following legitimate crosses (Trognitz & Hermann, 2001). In contrast, illegitimate crosses produced significantly less seeds than legitimate crosses in *O. hirta* var. *tubiflora* at Vanrhynsdorp. The Vanrhynsdorp population reportedly has the highest ploidy level (6x) of the three studied here. Interestingly, the seeds produced through both legitimate and illegitimate crosses in this population displayed pleiocotyly, often forming more than two cotyledons (3 and 4). Some eudicot genera do present variation in cotyledon number and which usually are a result from genetic mutations (Chandler, 2007). Additional cotyledons may confer an enhancement in seedling establishment, increasing the photosynthetic surface area and therefore potential productivity (Chandler, 2007). Despite the incompatibility relaxation and the high ploidy level, this population did not display any morphological deviation from the trimorphic state. This contrasts to what was found in *Primula* and *Aleuritia*, in which homostyly was prevalent in polyploid lineages. Like in the Turneraceae, this population of *O. hirta* var. *tubiflora* showed no correlation between breeding system and polyploidy (Shore *et al.*, 2006). In general, sexual reproductive organs were shorter than in the other two populations, but they were still reciprocally arranged, presenting the highest reciprocity index of all populations studied. Pollen grain sizes were also distinctly trimorphic. There is thus no evidence for the relaxation of self-incompatibility and the influence of polyploidy on self-incompatibility should be investigated further.

In general, seed set in all three populations was low, as it was limited to one third of the seed production potential of an *O. hirta* var. *tubiflora* flower. The highest seed set obtained from any cross in this pollination experiment was 7 seeds per capsule, while the average seed set after legitimate pollination for all populations was 2.6 seeds per capsule. The fertility rate in *O. hirta* var. *tubiflora* should be determined before further investigation of the possible causes of limited

seed formation can be explored. This variety displays many morphological characteristics that suggest non-generalist pollination and seeds are produced in the field, but levels of natural seed production still remain unknown.

Despite the limited distribution, the different populations of *O. hirta* var. *tubiflora* may be viewed as discrete units as they are separated by mountains and diverse ecological parameters that may isolate them fully (Linder, 2000). This offers a great opportunity to explore the individual expression of tristylly and polyploidy in each *O. hirta* var. *tubiflora* population in search of possible drivers governing the maintenance and/or breakdown of this system.

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1. Dissertation Structure
2. Tristyly in flowering plants: A review
3. Morph representation in <i>Oxalis</i> populations across the Cape Floristic Region
4. Tristyly and sexual organ morphology in South African <i>Oxalis</i>
5. Patterns related to morph frequency in tristylous <i>Oxalis</i> species
6. Three-dimensional reciprocity in <i>Oxalis</i>
7. Tristyly and self- incompatibility in a polyploid South African <i>Oxalis</i> species
8. Tristyly in the genus <i>Oxalis</i> in South Africa: A review

Introduction

This chapter summarises the findings of the five studies that explored the state of expression of the tristylous system in South African *Oxalis* species. The first study examined the ratio of floral morphs within populations, the second inspected sexual organ morphology and pollen morphology, the third compared patterns of morph expression between South African and American *Oxalis* species, the fourth evaluated three-dimensional tristily in *Oxalis* section *Sagittatae*, and the final study investigated the breeding system of *Oxalis hirta* var. *tubiflora*, a polyploid species. Collectively, these five studies dealt with the essence of tristily by analysing key elements that influence the pollination process and fertilization success in this system. This chapter also highlights essential characteristics that set tristily in South African *Oxalis* apart from other families and genera in which heterostyly is expressed.

The expression of tristily was investigated firstly through a study of morph equilibrium in Chapter 3. This regional-scale analysis of 104 populations included 58 species and explored the influence of geographic location and degree of clonality on the equilibrium of morphs at population level. In tristylous species with heteromorphic incompatibility, a 1:1:1 morph ratio is expected in large populations because of frequency-dependent mating, since full seed set is expected to be restricted to inter-morph crosses (Barrett, 1993). Other studies have indicated that environmental aspects influence this balance of floral morphs in populations, altering the function and maintenance of the tristylous system in different ways (Dupré & Ehrlén, 2002; Hodgins & Barrett, 2006, 2008a; Weller *et al.*, 2007). For this reason, explanatory variables assessed in this study included geographical location, levels of disturbance, vegetation type, vegetation structure, substrate type and soil moisture capacity. An isopletic equilibrium was not

found in all populations studied, with morph representation varying from the expected 1:1:1 ratio in 29% of populations analyzed, including populations that lack one or even two of the three floral morphs.

Anisoplety, the disequilibrium of floral morph representation in a population, is common among tristylous species. Studies on American *Oxalis* species have shown that they present predictable patterns of deviation on tristily expression. Firstly, and most commonly, they tend to lack the same morph type across populations, mainly the Mid morph (Marco & Arroyo, 1998; Weller, 1992). This has occurred in *Oxalis alpina* (Rose) Knuth and distily is considered derived from tristily in this species through loss of the Mid morph (Sosenski *et al.*, 2010). Lack of the same morph type across populations has also been found in species from different genera such as *Lythrum californicum* Torr. & Gray, *L. rotundifolia* L., *Pemphis acidula* J. R. & G., *Decodon verticillatus* (L.) Ell., *Eichhornia crassipes* (Mart.) Solms., and *Narcissus triandrus* (Barrett, 1977; Barrett *et al.*, 1989; Barrett *et al.*, 1997; Eckert & Barrett, 1993; Weller, 1992). This was not found to be the case among South African members of *Oxalis* (Chapter 5). The species varied in terms of the predominant morph type, and when morphs were lost, they could be any one of the three. Six populations of *O. glabra* were analyzed, of which Long morphs were the majority in three of them, while Mid morphs prevailed in the other three. Of the five *O. nidulans* populations analyzed, Long morphs were the majority in two populations, two other populations had Mid morphs abounding and one population was dominated by Short morphs (to the exclusion of the Long morph and near exclusion of the Mid morph). Similar non-directionality in terms of the morph that dominates and the morph that declines or is lost was also found in *O. pes-caprae* and *O. purpurea*.

The second pattern observed in many heterostylous species is that the variation in floral morph frequency follow a geographical pattern (Hodgins & Barrett, 2008a, Marco & Arroyo, 1998, Luo

et al., 2006; Weller *et al.*, 2007). For example, distylous populations of *O. alpina* in the Sky Islands in the Sonoran Desert are located towards northwest Arizona, while tristylous populations are concentrated in southeastern Arizona, New Mexico and Sonora (Weller *et al.*, 2007). Distylous populations of *Narcissus triandrus* occur in the northwestern Iberian Peninsula, while tristylous populations increased in frequency southeast of the Iberian Peninsula (Hodgins & Barrett, 2008a). Chapter 5 also explored the extent of geographical directionality in morph expression among southern African members of *Oxalis*, and results again contradicted findings in other tristylous species. There was no clear geographical pattern associated with anisoplety and/or the reduction and ultimate loss of any one of the morphs in any of the species studied.

It seems that tristily expression in South African *Oxalis* generally deviates at population level. Previously non-South African taxa like *O. alpina* and *N. triandrus*, have shown tristily breakdown in a broad geographical scale, but among South African *Oxalis* deviations in tristily expression appears to occur at a much smaller, more localized scale. In the Cape Floristic Region (CFR) of South Africa species are subject to spatial and environmental isolation due to considerable geographic and altitudinal variation brought about by the Cape Fold Mountains, exposing different populations of the same species to very different ecological pressures (Goldblatt & Manning, 2000, 2002; Linder, 2003). Linder (2003) highlights the various centres of endemism within the CFR, mostly separated by isolation and reproductive barriers. Gene flow across these barriers is believed to be limited by various factors, including different flowering times and pollinator specificity.

Within this scenario, it is reasonable to expect stochastic forces to have a greater influence on morph frequencies. Stochastic forces are factors related to the ecology and genetic make-up of populations by means of founder events and life history characteristics (Barrett, 1993). It is also

known that population size plays an important role by maximizing the influence that genetic drift will have on frequency-dependent selection, the dominant force maintaining the polymorphism (eg. *Eichhornia paniculata*). Population sizes of *Oxalis* species show considerable variation (pers. obs.). Some species occur in large, dense populations, while others are restricted to minute populations, usually specific to local, specialized environments.

In Chapter 4 different elements that influence pollen flow between different morphs within a species are explored through studies of the arrangement of sexual reproductive organ heights and pollen sizes. In order to understand the symmetry of mating in tristylous species, sexual reproductive organ heights and the relative distance between the three levels must be known (Hernández & Ornelas, 2007; Hodgins & Barrett, 2008b; Sanchez *et al.*, 2008). Lloyd *et al.* (1990) developed a method to quantify herkogamy in tristylous species. It has been successfully applied to non-South African *Oxalis* and other tristylous species and was thus also applied in this study (Marco & Arroyo, 1998; Lloyd *et al.* 1990). The analysis of 104 populations (representing 58 different species) indicated that flowers of most South African *Oxalis* species showed clear uniformity of organ levels. Sexual organs are borne at three distinct heights and are reciprocally arranged across the three floral morph types. In terms of this aspect of tristylous, most South African *Oxalis* species thus still fully conform to the typical morphological tristylous species (Lloyd & Webb, 1992; Barrett, 1993).

A number of species did, however, deviate from this pattern in very interesting ways. Several populations had morphs with no longer strictly three distinct reproductive organ levels. In these populations there were mismatches of reproductive organ heights, characterized mainly by differences in height of one level within one morph. For example, long-level anthers of Mid and Short morph flowers formed a ladder cascading down between the long-level and mid-level positions, while the mid-level organs remained of equal length. This type of mismatch was

identified in 11 different species. Interestingly, this morphological adjustment was not expressed in all populations of the species in which it was observed. *O. ciliaris* and *O. hirta*, for example, both only displayed this in one of the three populations studied. In *O. nidulans* two of five populations studied showed such mismatches, while it was only observed in one of the six *O. glabra* populations studied. Other types of mismatches were also identified. In some cases mid-level organs were positioned in very close proximity to long-level organs, or short-level organs in close proximity to mid-level organs. Such variations alter the symmetry of mating, and may lead to changes in morph frequencies in populations (Barrett, 1992; Barrett *et al.*, 1997, Hodgins & Barrett, 2008a, Weller *et al.*, 2007). Curiously no direct correlations were detected between sexual reproductive organ arrangement and morph frequency among the South African *Oxalis* populations studied, but this certainly merits further, more detailed investigation.

The final analysis in Chapter 4 focussed on the pollen size polymorphism associated with tristylous species. This is an important feature in terms of regulating disassortative pollen flow (Dulberger, 1992; Barrett *et al.*, 2000). In eight of the 15 populations analyzed, variation in pollen grain sizes followed variation in anther heights, with long level anthers producing the largest pollen grains, short level anthers producing the smallest pollen grains, and mid level anthers producing pollen grains of an intermediate size. The other seven populations studied showed interesting deviations in terms of the expression of this polymorphism. This incomplete pollen trimorphism did not show clear relations with morph ratios, ratio of organ heights or reciprocity indices. Observations in *O. hirta* var. *tubiflora* serve as a good example of this lack of association. The only anisoplectic population studied for this taxon had the highest reciprocity index and pollen grain sizes decreased normally from long- to short-level anthers. It seems as if pollen trimorphism is controlled independently from morph ratios, ratio of organ heights and the reciprocity indices, but this needs verification through more detailed and inclusive investigations.

Chapter 6 introduces a new variation of tristily, three-dimensional tristily, in both the Oxalidaceae and the angiosperms. The morphological and self-incompatibility expression of this system were investigated in two species represented by seven populations. Results exposed significant signs of breakdown of this new system. This was expressed through altered reciprocity of sexual reproductive organs and high levels of self-compatibility. Only one population (*O. nidulans*; Villiersdorp) presented deviation on the separation of reproductive organ levels and this population also was anisoplectic, while all populations studied presented self-compatibility among morphs. In this regard three-dimensional tristily differs from three-dimensional reciprocity, as no signs of breakdown have been detected in the latter (Armbruster *et al.*, 2006). Despite these signs of breakdown, intermorph pollen movement still seems to be relatively effective in some populations, given that they were still at isoplectic equilibrium. This equilibrium of morphs may result from the combination of reciprocity of organs in these populations and the still fully expressed pollen trimorphism.

In the final chapter the influence of polyploidy on tristily is explored in *O. hirta* var. *tubiflora*. This taxon displays a suite of attributes including unique pollen morphology, the production of aberrant pollen and different levels of polyploidy, which may affect its breeding system. The most significant finding was the very unusual widespread seed set in the two triploid populations. Artificial crossing experiments in three populations found polyploids to be fertile. All morphs were able to produce seeds after legitimate pollination and polyploidy did not interfere with the expression of tristily or the fertility of this taxon. There was, however, some relaxation in self-incompatibility in some of the populations.

Final observations

This study presents a broad-scale first attempt at understanding aspects of the tristily syndrome expressed among South African members of *Oxalis*. Results presented here offer exciting

opportunities for comparisons across tristylous genera and families. Additionally it offers a broad, solid platform from which research on the reproductive biology of *Oxalis* can, and should, be expanded upon. There are very few other examples of so many tristylous species within a single genus, and in which the genus is characterized by so many biological characters that may impact the expression of the syndrome.

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Appendix 1: *Oxalis* populations analyzed in the study of morph representation in South Africa.

Habitat details such as disturbance level, substrate, vegetation type and exposure are included. Additional information of any population and site such as minor and precise locality information, landscape, geology, soil moisture capacity, plant descriptions and herbarium samples can be obtained from the corresponding author.

Identification	Pop no	Collection Number	Collector	Major locality	Longitude	Latitude	Disturbance	Substrate	Vegetation type	Exposure
<i>O. adspersa</i>	6	0012	S.S.Siqueira & J. Zietsman	Gouda	S 33 22.207' E 19 00.833'		Very high	Clay soil	Farmland	Full sun
<i>O. adspersa</i>	12	0013	S.S.Siqueira & J. Zietsman	Citrusdal	S 32 41.210' E 18 56.548'		Very high	Gravel, soil and clay	Farmland	Full sun
<i>O. amblyodonta</i>	34	0007	L.L. Dreyer, J. Zietsman & F. Roets	Hermon	S 33 27.521' E 19 04.183'		Pristine	Sandy soil, rocks	Fynbos	Full sun
<i>O. amblyosepala</i>	23	0004	J. Zietsman	Vanrhynsdorp	S 31 46.678' E 18 45.960'		Pristine	Sand	Mountain Fynbos	Full sun
<i>O. aridicola</i>	41	0052	S.S.Siqueira & J. Zietsman	Clanwilliam	S 31 55.615' E 19 14.522'		Pristine	Red clay soil	Succulent Karoo	Full sun
<i>O. aurea</i>	16	0019	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 18.481' E 18 54.513'		Low	Clay soil	Renosterveld	Full sun
<i>O. callosa</i>	68	0012	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Nieuwoudtville	S 31 26.260' E 19 08.619'		Low	Bedrock - sandstone	Succulent Karoo	Full sun
<i>O. campicola</i>	40	0050	S.S.Siqueira & J. Zietsman	Calvinia	S 31 42.420' E 19 18.314'		Disturbed	Clay and small stones	Succulent Karoo	Full sun
<i>O. capillaceae</i> var. <i>tenata</i>	96	0008	S.S.Siqueira & B. Marais	Bredasdorp	S 34 28.513' E 20 25.813'		Fire	Sand and clay	Dune Fynbos	Full sun
<i>O. caprina</i>	2	0002	S.S.Siqueira & J. Zietsman	Caledon	S 34 07.019' E 19 50.471'		High	Sandy soil	Mountain Fynbos and Farmland	Partial shade
<i>O. caprina</i>	10	0004	S.S. Turketti & S.S.Siqueira	Caledon	S 34 07.019' E 19 50.471'		Very high	Organic-rich soil, gravel	Farmland	Partial shade

Identification	Pop no	Collection Number	Collector	Major locality	Longitude	Latitude	Disturbance	Substrate	Vegetation type	Exposure
<i>O. cf. fergusoniae</i>	87	0001	S.S. Siqueira, F. Roets & J. Zietsman	Robertson	S 33 47.462' E 19 47.002'		Low	Sandy soil	Robertson Karoo	Full sun
<i>O. cf. gracilipes</i>	50	0007	S.S. Turketti & S.S.Siqueira	Darling	S 33 28.451' E 18 27.184'		Very high	Clay and sand	Farmland	Full sun
<i>O. ciliaris</i>	75	0009	J. Zietsman	Mossel Bay	S 34 08.349' E 21 58.745'		Pristine	Sandy soil	Fynbos	Full sun
<i>O. ciliaris</i>	76	0010	J. Zietsman	Mossel Bay	S 34 08.944' E 21 58.011'		Disturbed	Sand and gravel	Fynbos	Full sun
<i>O. ciliaris</i>	94	0001	S.S.Siqueira & B. Marais	Bredasdorp	S 34 27.415' E 20 24.604'		Pristine	Sand and gravel	Dune Fynbos	Full sun
<i>O. compressa</i>	88	0123	K. Oberlander	Porterville	S 32 57.796' E 18 53.566'		High	Rocky soil	Farmland	Full sun
<i>O. comptonii</i>	27	0032	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 46.574' E 18 46.519'		Pristine	Clay and sand	Mountain Fynbos	Full sun
<i>O. densa</i>	54	0008	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Calvinia	S 31 15.730' E 19 29.793'		Pristine	Sandy soil	Succulent Karoo	Full sun
<i>O. depressa</i>	93	-	L.L. Dreyer	-	-	-	-	-	-	-
<i>O. depressa.</i>	92	0015	S.S. Turketti & S.S.Siqueira	Cape Agulhas	S 34 49.566' E 19 59.241'		Pristine	Sand and gravel	Mountain Fynbos	Shade/sun
<i>O. dines</i>	81	0121	K. Oberlander	Calvinia	S 31 47.677' E 19 16.590'		Low	Clay soil	Succulent Karoo	Full sun
<i>O. disticha</i>	89	0124	K. Oberlander	Porterville	S 32 56.691' E 18 50.629'		High	Rocky soil	Farmland	Full sun
<i>O. dregei</i>	59	2643b	Bytebier	Clanwilliam	S 32 04.205' E 19 04.522'		Disturbed	Sandy soil	Succulent Karoo	Full sun
<i>O. ebracteata</i>	14	0015	S.S.Siqueira & J. Zietsman	Citrusdal	S 32 38.921' E 18 57.063'		Low	Rocks and coarse sand	Fynbos	Full sun
<i>O. eckloniana</i>	8	0002	S.S. Turketti & S.S.Siqueira	Caledon	S 34 14.477' E 19 26.663'		Very high	Gravel soil	Town surroundings	Full sun
<i>O. engleriana</i>	53	0001	S.S. Siqueira, K. Oberlander & F. Roets	Villiersdorp	S 33 56.162' E 19 20.486'		Very high	Organic rich soil and gravel	Renosterveld	Partial shade
<i>O. flava</i>	19	0022	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 04.064' E 19 07.580'		Disturbed	Sandy soil	Renosterveld	Full sun

Identification	Pop no	Collection Number	Collector	Major locality	Longitude	Latitude	Disturbance	Substrate	Vegetation type	Exposure
<i>O. giftbergensis</i>	22	0003	J. Zietsman	Vanrhynsdorp	S 31 46.573' E 18 45.823'		Pristine	Gravel	Mountain Fynbos	Partial shade
<i>O. glabra</i>	3	0001	S.S.Siqueira & J. Zietsman	Caledon	S 34 08.354' E 19 43.567'		High	Sandy soil	Mountain Fynbos and Farmland	Shade/sun
<i>O. glabra</i>	30	0055	S.S.Siqueira & J. Zietsman	Hermon	S 33 26.820' E 19 02.890'		Pristine	Loamy soil	Renosterveld	Full sun
<i>O. glabra</i>	46	0003	S.S.Siqueira	Stellenbosch	S 33 55.964' E 18 52.563'		Disturbed	Sand and gravel	Fynbos	Full sun
<i>O. glabra</i>	49	0006	S.S. Turketti & S.S.Siqueira	Atlantis	S 33 40.395' E 18 36.453'		Very high	Organic-rich soil	Farmland	Partial shade
<i>O. glabra</i>	52	0002	S.S.Siqueira & C. Obone	Ceres	S 33 25.371' E 19 15.902'		Very high	Loamy soil	Farmland	Full sun
<i>O. glabra</i>	80	0005	S.S. Siqueira, K. Oberlander & F. Roets	Stellenbosch	S 33 52.681' E 18 48.095'		Very high	Loamy soil	Farmland	Full sun
<i>O. goniorrhiza</i>	58	0061	S.S.Siqueira & J. Zietsman	Ceres	S 33 20.637' E 19 21.986'		Disturbed	Stones and soil	Renosterveld	Full sun
<i>O. gracilis</i>	35	0034	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 43.043' E 18 46.113'		Pristine	Sandy soil	Renosterveld	Full sun
<i>O. heterophylla</i>	61	0002	S.S. Siqueira, K. Oberlander & F. Roets	Ceres	S 33 14.321' E 19 20.244'		Disturbed	Clay soil	Fynbos	Partial shade
<i>O. hirta</i>	4	0009	S.S.Siqueira & J. Zietsman	Malmesbury	S 33 24.580' E 18 22.891'		Very high	Bedrock - sandstone	Farmland	Partial shade
<i>O. hirta</i>	5	0002	S.S.Siqueira	Malmesbury	S 33 25.217' E 18 23.281'		Disturbed	Loamy soil	Renosterveld and Farmland	Full sun
<i>O. hirta</i>	32	0008	L.L. Dreyer, J. Zietsman & F. Roets	Hermon	S 33 26.926' E 19 04.206'		Pristine	Sandy soil	Renosterveld and Fynbos	Full sun
<i>O. hirta</i> var. <i>tenuicaulis</i>	104	0812	L.L. Dreyer	Langebaan	S 33 03.218' E 18 04.876'		Disturbed	Organic rich soil and sand	Town surroundings	Full sun
<i>O. hirta</i> var. <i>tubiflora</i>	15	0017	S.S.Siqueira & J. Zietsman	Citrusdal	S 32 37.786' E 18 56.797'		Disturbed	Sandy soil	Renosterveld	Full sun

Identification	Pop no	Collection Number	Collector	Major locality	Longitude	Latitude	Disturbance	Substrate	Vegetation type	Exposure
<i>O. hirta</i> var. <i>tubiflora</i>	21	0025	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 08.885' E	18 56.504'	Pristine	Sandy soil	Fynbos	Full sun
<i>O. hirta</i> var. <i>tubiflora</i>	28	0033	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 43.859' E	18 46.432'	Disturbed	Sandy soil	Renosterveld	Shade/sun
<i>O. incarnata</i>	66	0005	S.S.Siqueira	Cape Town	S 33 59.097' E	18 26.060'	Low	Loamy soil	Closed forest	Shade
<i>O. incarnata</i>	90	0013	S.S. Turketti & S.S.Siqueira	Betty's Bay	S 34 20.047' E	18 56.902'	Pristine	Sand and gravel	Closed forest	Shade
<i>O. lanata</i>	63	0004	S.S. Siqueira, K. Oberlander & F. Roets	Ceres	S 33 14.321' E	19 20.244'	High	Sandy soil	Fynbos	Full sun
<i>O. livida</i>	74	0008	J. Zietsman	Riversdale	S 34 09.732' E	21 18.468'	Disturbed	Clay soil	Renosterveld	Full sun
<i>O. luteola</i>	33	0933	D.U.B.	Hermon	S 33 26.926' E	19 04.206'	Pristine	Gravel	Fynbos	Full sun
<i>O. massoniana</i>	67	0011	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Nieuwoudtville	S 31 15.916' E	19 03.464'	Disturbed	Sandy soil	Farmland	Full sun
<i>O. monophylla</i>	97	0006	S.S.Siqueira	Stellenbosch	S 33 55.982' E	18 52.378'	Pristine	Clay and sand	Fynbos	Partial shade
<i>O. multicaulis</i>	79	0007	S.S. Siqueira, K. Oberlander & F. Roets	Stellenbosch	S 33 52.681' E	18 48.095'	Very high	Loamy soil	Farmland	Full sun
<i>O. natans</i>	64	0160	K. Oberlander	Stellenbosch	S 34 01.099' E	18 48.326'	Disturbed	Clay soil	Farmland	Full sun
<i>O. natans</i>	65	0006	J. Zietsman	Hermon	S 33 25.334' E	19 01.673'	Pristine	Sandy soil	Renosterveld	Full sun
<i>O. nidulans</i>	83	-	K. Oberlander	-	-	-	-	-	-	-
<i>O. nidulans</i>	95	0003	S.S.Siqueira & B. Marais	Bredasdorp	S 34 28.513' E	20 25.813'	Fire	Sand and gravel	Dune Fynbos	Full sun
<i>O. nidulans</i>	98	0001	L.L. Dreyer, K.J. Esler & S.S. Siqueira	Villiersdorp	S 33 57.761' E	19 30.575'	Pristine	Clay and sand	Renosterveld	Shade
<i>O. nidulans</i>	101	0608	L.L. Dreyer	Hermon	S 33 26.820' E	19 02.890'	Pristine	Sand and gravel	Renosterveld	Full sun
<i>O. nidulans</i>	103	0607	L.L. Dreyer	Hermon	S 33 26.820' E	19 02.890'	Pristine	Sand and gravel	Renosterveld	Full sun

Identification	Pop no	Collection Number	Collector	Major locality	Longitude	Latitude	Disturbance	Substrate	Vegetation type	Exposure
<i>O. nidulans</i> var. <i>denticulata</i>	31	0001	L.L. Dreyer, J. Zietsman & F. Roets	Hermon	S 33 26.820' E 19 02.890'		Pristine	Sandy soil	Renosterveld	Full sun
<i>O. obtusa</i>	38	0041	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 46.574' E 18 46.519'		Pristine	Clay and sand	Mountain Fynbos	Full sun
<i>O. obtusa</i>	55	0004	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Calvinia	S 31 28.189' E 19 42.052'		Disturbed	Clay and soil	Succulent Karoo	Full sun
<i>O. obtusa</i>	62	0003	S.S. Siqueira, K. Oberlander & F. Roets	Ceres	S 33 14.321' E 19 20.244'		Disturbed	Clay soil	Fynbos	Full sun
<i>O. oculifera</i>	26	0005	J. Zietsman	Vanrhynsdorp	S 31 46.912' E 18 46.011'		Pristine	Fine Clay soil	Mountain Fynbos	Full sun
<i>O. oligophylla</i>	60	0042	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 45.922' E 18 46.164'		Pristine	Dark sand and rocks	Renosterveld	Partial shade
<i>O. oreophila</i>	18	0002	J. Zietsman	Clanwilliam	S 32 08.932' E 19 01.761'		Pristine	Clay soil	Renosterveld	Full sun
<i>O. pes-caprae</i>	7	0001	S.S. Turketti & S.S.Siqueira	Villiersdorp	S 34 04.883' E 19 16.218'		Very high	Clay soil	Town surroundings and Renosterveld	Full sun
<i>O. pes-caprae</i>	29	0054	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 46.678' E 18 45.960'		Low	Sandy soil	Mountain Fynbos and Farmland	Full sun
<i>O. pes-caprae</i>	37	0056	S.S.Siqueira & J. Zietsman	Nuwerus	S 31 14.952' E 18 17.946'		Disturbed	Sand and stones	Succulent Karoo	Full sun
<i>O. pes-caprae</i>	51	0001	S.S.Siqueira & C. Obone	Ceres	S 33 29.564' E 19 11.710'		Very high	Gravel soil	Farmland	Full sun
<i>O. pes-caprae</i>	57	0060	S.S.Siqueira & J. Zietsman	Touws River	S 33 12.295' E 19 43.812'		Pristine	Clay soil	Tanqua karoo	Shade/sun
<i>O. polyphylla</i>	77	0109	Christian van Schalkwyk	Mossel Bay	S 34 08.944' E 21 58.011'		Disturbed	Sand and gravel	Fynbos	Full sun
<i>O. pulchella</i>	100	0002	L.L. Dreyer, K.J. Esler & S.S. Siqueira	Villiersdorp	S 33 56.468' E 19 31.255'		Pristine	Clay and sand	Mountain Fynbos	Full sun
<i>O. purpurea</i> (pink)	47	0059	S.S.Siqueira & J. Zietsman	Malmesbury	S 33 26.573' E 18 36.592'		Very high	Gravel	Farmland	Full sun

Identification	Pop no	Collection Number	Collector	Major locality	Longitude	Latitude	Disturbance	Substrate	Vegetation type	Exposure
<i>O. purpurea</i> (white)	25	0030	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 46.573' E	18 45.823'	Disturbed	Gravel	Fynbos	Full sun
<i>O. purpurea</i> (white)	42	0057	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 01.774' E	19 11.523'	Disturbed	Organic rich soil and sand	Succulent Karoo	Full sun
<i>O. purpurea</i> (yellow)	11	0011	J. Zietsman	Citrusdal	S 32 38.921' E	18 57.063'	High	Gravel	Fynbos	Partial shade
<i>O. purpurea</i> (yellow)	36	0038	S.S.Siqueira & J. Zietsman	Nuwerus	S 31 14.392' E	18 32.146'	Low	Sand and stones	Succulent Karoo	Full sun
<i>O. purpurea</i> (yellow)	43	0058	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 01.774' E	19 11.523'	Disturbed	Organic rich soil and sand	Succulent Karoo	Full sun
<i>O. purpurea</i> (yellow)	56	0009	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Calvinia	S 31 28.189' E	19 42.052'	Disturbed	Sand and gravel	Succulent Karoo	Full sun
<i>O. purpurea</i> (yellow)	70	0009	S.S. Turketti & S.S.Siqueira	Villiersdorp	S 33 58.938' E	19 10.701'	Disturbed	Clay soil	Renosterveld	Full sun
<i>O. recticaulis</i>	82	-	K. Oberlander	-	-	-	-	-	-	-
<i>O. salteri</i>	39	0045	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 41.817' E	18 45.598'	Low	Sand and gravel	Succulent Karoo	Full sun
<i>O. sp.</i>	71	0010	S.S. Turketti & S.S.Siqueira	Franschhoek	S 33 55.045' E	19 09.522'	Pristine	Sandy soil	Mountain Fynbos	Full sun
<i>O. sp.</i>	72	0011	S.S. Turketti & S.S.Siqueira	Villiersdorp	S 34 01.313' E	19 13.500'	Low	Sandy soil	Fynbos	Full sun
<i>O. sp.</i>	73	0012	S.S. Turketti & S.S.Siqueira	Villiersdorp	S 33 58.938' E	19 10.701'	Disturbed	Sandy soil	Renosterveld	Full sun
<i>O. sp.</i>	99	0003	L.L. Dreyer, K.J. Esler & S.S. Siqueira	Villiersdorp	S 33 57.761' E	19 30.575'	Pristine	Clay and sand	Renosterveld	Shade
<i>O. sp.</i>	102	0606	L.L. Dreyer	Hermon	S 33 26.926' E	19 04.206'	Pristine	Sand and gravel	Renosterveld	Full sun
<i>O. stellata</i>	91	0014	S.S. Turketti & S.S.Siqueira	Cape Agulhas	S 34 48.667' E	20 03.338'	Low	Sand	Dune Fynbos	Full sun

Identification	Pop no	Collection Number	Collector	Major locality	Longitude	Latitude	Disturbance	Substrate	Vegetation type	Exposure
<i>O. suteroides</i>	24	0026	S.S.Siqueira & J. Zietsman	Nieuwoudtville	S 31 20.277' E 19 15.563'		Pristine	Clay soil	Renosterveld	Full sun
<i>O. suteroides</i>	69	0013	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Nieuwoudtville	S 31 26.260' E 19 08.619'		Low	Bedrock - sandstone	Succulent Karoo	Full sun
<i>O. tenella</i>	17	0020	S.S.Siqueira & J. Zietsman	Citrusdal	S 32 18.481' E 18 54.513'		Low	Clay soil	Renosterveld	Full sun
<i>O. tenella</i>	20	0023	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 05.519' E 19 03.783'		Pristine	Sandy soil	Renosterveld	Partial shade
<i>O. tenuifolia</i>	45	0004	S.S.Siqueira	Stellenbosch	S 33 55.982' E 18 52.378'		Disturbed	Sand and gravel	Fynbos	Partial shade
<i>O. tenuifolia</i>	48	0005	S.S. Turketti & S.S.Siqueira	Malmesbury	S 33 30.036' E 18 39.833'		Very high	Organic-rich soil and gravel	Farmland	Full sun
<i>O. tomentosa</i>	84	0001	J. Zietsman	Malmesbury	S 33 25.217' E 18 23.281'		Low	Sandy soil	Renosterveld	Full sun
<i>O. tomentosa</i>	85	0012	J. Zietsman	Saron	S 33 11.305' E 18 59.060'		Very high	Clay soil	Renosterveld	Full sun
<i>O. tomentosa</i>	86	0003	Informal collector K.J. Esler	Hermon	S 33 25.607' E 19 02.762'		Pristine	Clay soil	Renosterveld	Full sun
<i>O. truncatula</i>	1	0001	S.S.Siqueira	Caledon	S 34 07.019' E 19 50.471'		High	Sandy soil	Mountain Fynbos and Farmland	Partial shade
<i>O. versicolor</i>	78	0006	S.S. Siqueira, K. Oberlander & F. Roets	Stellenbosch	S 33 52.681' E 18 48.095'		Very high	Loamy soil	Farmland	Full sun
<i>O. viscosa</i>	13	0014	S.S.Siqueira & J. Zietsman	Citrusdal	S 32 39.223' E 18 57.321'		Disturbed	Sandy soil	Fynbos	Partial shade
<i>O. xantha</i>	44	0053	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 01.774' E 19 11.523'		Disturbed	Red sand and clay	Succulent Karoo	Full sun
<i>O. zeekoevleyensis</i>	9	0003	S.S. Turketti & S.S.Siqueira	Caledon	S 34 16.611' E 19 31.452'		Very high	Organic-rich soil	Farmland	Full sun

Appendix 2: *Oxalis* populations sampled for the analysis of reproductive organ heights in South Africa.

Locality data is given in terms of longitude and latitude in degrees and decimal minutes. Percentages of Long (L), Mid (M) and Short (S) morphs and isoplethic equilibrium (IE) were based on the total number of flowers sampled. G value is the likelihood between sampled and expected morph frequencies. Organ height ratios were obtained by dividing the average mid- and long-level organs by the average short-level organs. Lloyd's reciprocity index (RI) values were calculated by dividing the average distance between adjacent levels for the average difference within levels (Lloyd et al., 1990). Larger values indicate higher reciprocity.

Identification	Pop no	Collection Number	Collector	Major locality ^a	Longitude	Latitude	% L	% M	% S	IE	G	Organ height ratios	RI
<i>O. adspersa</i>	6	0012	S.S.Siqueira & J. Zietsman	Gouda	S 33 22.207'	E 19 00.833'	33.0	21.7	45.3	*	9.00	1:1.6:2.5	6.9
<i>O. adspersa</i>	12	0013	S.S.Siqueira & J. Zietsman	Citrusdal	S 32 41.210'	E 18 56.548'	27.3	30.9	41.8	ns	3.69	1:1.6:2.4	13.9
<i>O. amblyodonta</i>	34	0007	L.L. Dreyer, J. Zietsman & F. Roets	Hermon	S 33 27.521'	E 19 04.183'	24.7	37.6	37.6	ns	3.00	1:1.4:2.1	10.9
<i>O. amblyosepala</i>	23	0004	J. Zietsman	Vanrhynsdorp	S 31 46.678'	E 18 45.960'	31.4	35.2	33.3	ns	0.23	1:1.6:2.8	9.0
<i>O. aridicola</i>	41	0052	S.S.Siqueira & J. Zietsman	Clanwilliam	S 31 55.615'	E 19 14.522'	35.7	31.0	33.3	ns	0.43	1:1.4:1.9	8.8
<i>O. aurea</i>	16	0019	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 18.481'	E 18 54.513'	30.6	36.0	33.3	ns	0.49	1:1.8:2.8	8.6
<i>O. callosa</i>	68	0012	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Nieuwoudtville	S 31 26.260'	E 19 08.619'	25.3	45.5	29.3	*	6.54	1:1.6:2.3	5.7
<i>O. campicola</i>	40	0050	S.S.Siqueira & J. Zietsman	Calvinia	S 31 42.420'	E 19 18.314'	32.7	34.5	32.7	ns	0.07	1:1.4:1.9	6.9
<i>O. capillaceae</i> var. <i>tenata</i>	96	0008	S.S.Siqueira & B. Marais	Bredasdorp	S 34 28.513'	E 20 25.813'	29.6	38.0	32.4	ns	1.15	1:1.4:2.1	7.7
<i>O. caprina</i>	2	0002	S.S.Siqueira & J. Zietsman	Caledon	S 34 07.019'	E 19 50.471'	0.0	100.0	0.0	**	103.26	1:1.5:2.1	na
<i>O. caprina</i>	10	0004	S.S. Turketti & S.S.Siqueira	Caledon	S 34 07.019'	E 19 50.471'	0.8	0.0	99.2	**	260.82	1:1.3:2.1	na
<i>O. cf. fergusoniae</i>	87	0001	S.S. Siqueira, F. Roets & J. Zietsman	Robertson	S 33 47.462'	E 19 47.002'	0.0	6.3	93.7	**	136.30	1:1.4:1.9	na

Identification	Pop no	Collection Number	Collector	Major locality ^a	Longitude	Latitude	% L	% M	% S	IE	G	Organ height ratios	RI
<i>O. cf. gracilipes</i>	50	0007	S.S. Turketti & S.S. Siqueira	Darling	S 33 28.451'	E 18 27.184'	36.8	43.2	20.0	*	8.78	1:1.4:2.1	5.1
<i>O. ciliaris</i>	75	0009	J. Zietsman	Mossel Bay	S 34 08.349'	E 21 58.745'	48.5	31.7	19.8	**	12.70	1:1.5:2.1	7.6
<i>O. ciliaris</i>	76	0010	J. Zietsman	Mossel Bay	S 34 08.944'	E 21 58.011'	28.8	43.2	27.9	ns	4.73	1:1.5:2.2	5.5
<i>O. ciliaris</i>	94	0001	S.S. Siqueira & B. Marais	Bredasdorp	S 34 27.415'	E 20 24.604'	27.8	38.9	33.3	ns	2.01	1:1.5:2.1	7.5
<i>O. compressa</i>	88	0123	K. Oberlander	Porterville	S 32 57.796'	E 18 53.566'	36.1	36.1	27.8	ns	1.55	1:1.4:2.0	17.2
<i>O. comptonii</i>	27	0032	S.S. Siqueira & J. Zietsman	Vanrhynsdorp	S 31 46.574'	E 18 46.519'	27.5	21.6	51.0	*	7.08	1:1.5:2.2	8.4
<i>O. densa</i>	54	0008	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Calvinia	S 31 15.730'	E 19 29.793'	33.0	23.0	44.0	*	6.70	1:1.5:2.3	11.0
<i>O. depressa</i>	93	-	L.L. Dreyer	-	-	-	38.6	25.0	36.4	ns	2.95	1:1.6:2.6	28.2
<i>O. depressa.</i>	92	0015	S.S. Turketti & S.S. Siqueira	Cape Agulhas	S 34 49.566'	E 19 59.241'	32.1	33.0	34.9	ns	0.13	1:1.6:2.5	12.7
<i>O. dines</i>	81	0121	K. Oberlander	Calvinia	S 31 47.677'	E 19 16.590'	31.4	34.7	33.9	ns	0.22	1:1.5:2.2	5.1
<i>O. disticha</i>	89	0124	K. Oberlander	Porterville	S 32 56.691'	E 18 50.629'	36.0	39.5	24.6	ns	4.35	1:1.5:2.1	7.0
<i>O. dregei</i>	59	2643b	Bytebier	Clanwilliam	S 32 04.205'	E 19 04.522'	30.6	17.1	52.3	**	21.07	1:1.5:2.2	12.7
<i>O. ebracteata</i>	14	0015	S.S. Siqueira & J. Zietsman	Citrusdal	S 32 38.921'	E 18 57.063'	26.8	36.1	37.1	ns	1.95	1:1.5:2.2	7.2
<i>O. eckloniana</i>	8	0002	S.S. Turketti & S.S. Siqueira	Caledon	S 34 14.477'	E 19 26.663'	31.5	35.2	33.3	ns	0.22	1:1.3:1.7	5.4
<i>O. engleriana</i>	53	0001	S.S. Siqueira, K. Oberlander & F. Roets	Villiersdorp	S 33 56.162'	E 19 20.486'	30.4	28.4	41.2	ns	2.80	1:1.4:2.4	7.6
<i>O. flava</i>	19	0022	S.S. Siqueira & J. Zietsman	Clanwilliam	S 32 04.064'	E 19 07.580'	31.7	28.8	39.4	ns	1.83	1:1.5:2.3	7.1
<i>O. giftbergensis</i>	22	0003	J. Zietsman	Vanrhynsdorp	S 31 46.573'	E 18 45.823'	31.7	38.5	29.8	ns	1.27	1:1.4:2.2	7.9

Identification	Pop no	Collection Number	Collector	Major locality ^a	Longitude	Latitude	% L	% M	% S	IE	G	Organ height ratios	RI
<i>O. glabra</i>	3	0001	S.S.Siqueira & J. Zietsman	Caledon	S 34 08.354'	E 19 43.567'	0.0	83.9	16.1	**	40.72	1:1.1:1.7	na
<i>O. glabra</i>	30	0055	S.S.Siqueira & J. Zietsman	Hermon	S 33 26.820'	E 19 02.890'	41.8	23.5	34.7	ns	5.21	1:1.5:2.3	6.0
<i>O. glabra</i>	46	0003	S.S.Siqueira	Stellenbosch	S 33 55.964'	E 18 52.563'	38.9	30.5	30.5	ns	1.80	1:1.4:2.3	12.0
<i>O. glabra</i>	49	0006	S.S. Turketti & S.S.Siqueira	Atlantis	S 33 40.395'	E 18 36.453'	29.0	42.0	29.0	ns	3.26	1:1.4:2.3	25.3
<i>O. glabra</i>	52	0002	S.S.Siqueira & C. Obone	Ceres	S 33 25.371'	E 19 15.902'	58.7	26.9	14.4	**	31.85	1:1.4:2.1	3.6
<i>O. glabra</i>	80	0005	S.S. Siqueira, K. Oberlander & F. Roets	Stellenbosch	S 33 52.681'	E 18 48.095'	29.5	39.7	30.8	ns	1.42	1:1.5:2.4	18.2
<i>O. goniorrhiza</i>	58	0061	S.S.Siqueira & J. Zietsman	Ceres	S 33 20.637'	E 19 21.986'	31.8	33.6	34.5	ns	0.13	1:1.5:2.5	15.6
<i>O. gracilis</i>	35	0034	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 43.043'	E 18 46.113'	31.5	29.6	38.9	ns	1.52	1:1.4:2.2	5.1
<i>O. heterophylla</i>	61	0002	S.S. Siqueira, K. Oberlander & F. Roets	Ceres	S 33 14.321'	E 19 20.244'	34.9	29.1	36.0	ns	0.74	1:1.3:1.9	7.3
<i>O. hirta</i>	4	0009	S.S.Siqueira & J. Zietsman	Malmesbury	S 33 24.580'	E 18 22.891'	35.3	32.3	32.3	ns	0.24	1:1.6:2.4	19.5
<i>O. hirta</i>	5	0002	S.S.Siqueira	Malmesbury	S 33 25.217'	E 18 23.281'	39.0	28.0	33.0	ns	1.82	1:1.4:2.0	10.1
<i>O. hirta</i>	32	0008	L.L. Dreyer, J. Zietsman & F. Roets	Hermon	S 33 26.926'	E 19 04.206'	27.6	23.7	48.7	*	7.85	1:1.5:2.1	12.4
<i>O. hirta</i> var. <i>tenuicaulis</i>	104	0812	L.L. Dreyer	Langebaan	S 33 03.218'	E 18 04.876'	26.7	26.7	46.5	*	7.53	1:1.3:1.7	5.9
<i>O. hirta</i> var. <i>tubiflora</i>	15	0017	S.S.Siqueira & J. Zietsman	Citrusdal	S 32 37.786'	E 18 56.797'	39.4	28.3	32.3	ns	1.86	1:1.4:1.8	5.1
<i>O. hirta</i> var. <i>tubiflora</i>	21	0025	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 08.885'	E 18 56.504'	30.2	35.8	34.0	ns	0.53	1:1.5:2.0	4.7
<i>O. hirta</i> var. <i>tubiflora</i>	28	0033	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 43.859'	E 18 46.432'	33.3	44.4	22.2	*	6.80	1:1.4:1.8	6.3
<i>O. incarnata</i>	66	0005	S.S.Siqueira	Cape Town	S 33 59.097'	E 18 26.060'	65.3	34.7	0.0	**	91.57	1:1.5:2.4	na

Identification	Pop no	Collection Number	Collector	Major locality ^a	Longitude	Latitude	% L	% M	% S	IE	G	Organ height ratios	RI
<i>O. incarnata</i>	90	0013	S.S. Turketti & S.S. Siqueira	Betty's Bay	S 34 20.047'	E 18 56.902'	100.0	0.0	0.0	**	54.93	1:1.4:2.3	na
<i>O. lanata</i>	63	0004	S.S. Siqueira, K. Oberlander & F. Roets	Ceres	S 33 14.321'	E 19 20.244'	6.7	0.0	93.3	**	153.66	1:1.3:1.9	na
<i>O. livida</i>	74	0008	J. Zietsman	Riversdale	S 34 09.732'	E 21 18.468'	33.9	28.1	38.0	ns	1.82	1:1.5:2.5	10.5
<i>O. luteola</i>	33	0933	D.U.B.	Hermon	S 33 26.926'	E 19 04.206'	34.0	33.0	33.0	ns	0.02	1:1.4:2.5	4.3
<i>O. massoniana</i>	67	0011	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Nieuwoudtville	S 31 15.916'	E 19 03.464'	35.9	40.0	24.1	*	6.16	1:1.4:2.0	9.9
<i>O. monophylla</i>	97	0006	S.S. Siqueira	Stellenbosch	S 33 55.982'	E 18 52.378'	32.0	38.0	30.0	ns	1.02	1:1.7:2.7	24.1
<i>O. multicaulis</i>	79	0007	S.S. Siqueira, K. Oberlander & F. Roets	Stellenbosch	S 33 52.681'	E 18 48.095'	25.5	39.4	35.1	ns	2.92	1:1.5:2.2	18.5
<i>O. natans</i>	64	0160	K. Oberlander	Stellenbosch	S 34 01.099'	E 18 48.326'	39.0	31.0	30.0	ns	1.43	1:1.4:1.9	3.9
<i>O. natans</i>	65	0006	J. Zietsman	Hermon	S 33 25.334'	E 19 01.673'	27.9	27.9	44.2	ns	4.36	1:1.4:2.0	6.8
<i>O. nidulans</i>	83	-	K. Oberlander	-	-	-	31.6	37.8	30.6	ns	0.86	1:1.3:1.5	9.1
<i>O. nidulans</i>	95	0003	S.S. Siqueira & B. Marais	Bredasdorp	S 34 28.513'	E 20 25.813'	35.4	32.3	32.3	ns	0.18	1:1.2:1.6	5.0
<i>O. nidulans</i>	98	0001	L.L. Dreyer, K.J. Esler & S.S. Siqueira	Villiersdorp	S 33 57.761'	E 19 30.575'	0.0	3.0	97.0	**	317.73	1:1.1:1.3	na
<i>O. nidulans</i>	101	0608	L.L. Dreyer	Hermon	S 33 26.820'	E 19 02.890'	36.0	29.0	35.0	ns	0.88	1:1.5:2.4	8.4
<i>O. nidulans</i>	103	0607	L.L. Dreyer	Hermon	S 33 26.820'	E 19 02.890'	35.2	36.2	28.6	ns	1.11	1:1.4:1.8	5.0
<i>O. nidulans</i> var. <i>denticulata</i>	31	0001	L.L. Dreyer, J. Zietsman & F. Roets	Hermon	S 33 26.820'	E 19 02.890'	35.0	44.0	21.0	*	8.44	1:1.4:1.8	6.3
<i>O. obtusa</i>	38	0041	S.S. Siqueira & J. Zietsman	Vanrhynsdorp	S 31 46.574'	E 18 46.519'	43.5	28.3	28.3	ns	4.09	1:1.5:2.4	5.6
<i>O. obtusa</i>	55	0004	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Calvinia	S 31 28.189'	E 19 42.052'	40.2	29.9	29.9	ns	1.81	1:1.4:2.1	16.3

Identification	Pop no	Collection Number	Collector	Major locality ^a	Longitude	Latitude	% L	% M	% S	IE	G	Organ height ratios	RI
<i>O. obtusa</i>	62	0003	S.S. Siqueira, K. Oberlander & F. Roets	Ceres	S 33 14.321' E 19 20.244'	34.3	37.4	28.3	ns	1.30	1:1.4:2.2	8.6	
<i>O. oculifera</i>	26	0005	J. Zietsman	Vanrhynsdorp	S 31 46.912' E 18 46.011'	25.7	46.8	27.5	*	8.51	1:1.7:2.6	10.6	
<i>O. oligophylla</i>	60	0042	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 45.922' E 18 46.164'	21.4	46.4	32.1	ns	5.32	1:1.4:2.1	14.2	
<i>O. oreophila</i>	18	0002	J. Zietsman	Clanwilliam	S 32 08.932' E 19 01.761'	29.8	28.7	41.5	ns	2.74	1:1.7:2.4	5.7	
<i>O. pes-caprae</i>	7	0001	S.S. Turketti & S.S.Siqueira	Villiersdorp	S 34 04.883' E 19 16.218'	24.3	12.6	63.1	**	45.03	1:1.5:2.3	5.6	
<i>O. pes-caprae</i>	29	0054	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 46.678' E 18 45.960'	35.6	37.5	26.9	ns	2.05	1:1.4:2.3	13.5	
<i>O. pes-caprae</i>	37	0056	S.S.Siqueira & J. Zietsman	Nuwerus	S 31 14.952' E 18 17.946'	27.5	43.1	29.4	ns	4.31	1:1.4:2.1	40.7	
<i>O. pes-caprae</i>	51	0001	S.S.Siqueira & C. Obone	Ceres	S 33 29.564' E 19 11.710'	38.8	36.9	24.3	ns	4.07	1:1.4:2.2	7.3	
<i>O. pes-caprae</i>	57	0060	S.S.Siqueira & J. Zietsman	Touws River	S 33 12.295' E 19 43.812'	28.6	46.2	25.2	*	8.71	1:1.4:2.0	9.6	
<i>O. polyphylla</i>	77	0109	Christian van Schalkwyk	Mossel Bay	S 34 08.944' E 21 58.011'	25.7	28.4	45.9	*	7.50	1:1.6:2.4	9.2	
<i>O. pulchella</i>	100	0002	L.L. Dreyer, K.J. Esler & S.S. Siqueira	Villiersdorp	S 33 56.468' E 19 31.255'	37.8	24.5	37.8	ns	3.64	1:1.4:2.2	7.5	
<i>O. purpurea</i> (pink)	47	0059	S.S.Siqueira & J. Zietsman	Malmesbury	S 33 26.573' E 18 36.592'	1.0	6.9	92.2	**	162.00	1:1.4:2.2	4.0	
<i>O. purpurea</i> (white)	25	0030	S.S.Siqueira & J. Zietsman	Vanrhynsdorp	S 31 46.573' E 18 45.823'	33.0	40.2	26.8	ns	2.62	1:1.4:2.3	5.6	
<i>O. purpurea</i> (white)	42	0057	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 01.774' E 19 11.523'	34.3	37.1	28.6	ns	1.22	1:1.4:2.3	8.3	
<i>O. purpurea</i> (yellow)	11	0011	J. Zietsman	Citrusdal	S 32 38.921' E 18 57.063'	32.4	41.2	26.5	ns	3.33	1:1.4:2.3	6.2	
<i>O. purpurea</i> (yellow)	36	0038	S.S.Siqueira & J. Zietsman	Nuwerus	S 31 14.392' E 18 32.146'	37.9	29.1	33.0	ns	1.18	1:1.4:2.4	11.0	
<i>O. purpurea</i> (yellow)	43	0058	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 01.774' E 19 11.523'	34.0	38.0	28.0	ns	1.54	1:1.4:2.3	6.2	

Identification	Pop no	Collection Number	Collector	Major locality ^a	Longitude	Latitude	% L	% M	% S	IE	G	Organ height ratios	RI
<i>O. purpurea</i> (yellow)	56	0009	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Calvinia	S 31 28.189'	E 19 42.052'	38.6	25.7	35.6	ns	2.86	1:1.4:2.0	5.3
<i>O. purpurea</i> (yellow)	70	0009	S.S. Turketti & S.S. Siqueira	Villiersdorp	S 33 58.938'	E 19 10.701'	36.5	40.4	23.1	ns	2.72	1:1.4:2.3	7.7
<i>O. recticaulis</i>	82	-	K. Oberlander	-	-	-	66.7	18.8	14.6	**	22.43	1:1.5:2.2	10.2
<i>O. salteri</i>	39	0045	S.S. Siqueira & J. Zietsman	Vanrhynsdorp	S 31 41.817'	E 18 45.598'	30.4	35.3	34.3	ns	0.42	1:1.4:2.2	13.8
<i>O. sp.</i>	71	0010	S.S. Turketti & S.S. Siqueira	Franschhoek	S 33 55.045'	E 19 09.522'	41.1	33.3	25.6	ns	3.30	1:1.4:2.1	10.3
<i>O. sp.</i>	72	0011	S.S. Turketti & S.S. Siqueira	Villiersdorp	S 34 01.313'	E 19 13.500'	21.6	38.2	40.2	*	6.90	1:1.4:2.1	11.1
<i>O. sp.</i>	73	0012	S.S. Turketti & S.S. Siqueira	Villiersdorp	S 33 58.938'	E 19 10.701'	28.1	41.7	30.2	ns	2.97	1:1.6:2.3	7.0
<i>O. sp.</i>	99	0003	L.L. Dreyer, K.J. Esler & S.S. Siqueira	Villiersdorp	S 33 57.761'	E 19 30.575'	34.4	30.0	35.6	ns	0.95	1:1.3:2.0	8.1
<i>O. sp.</i>	102	0606	L.L. Dreyer	Hermon	S 33 26.926'	E 19 04.206'	41.3	28.9	29.8	ns	3.37	1:1.4:2.0	14.7
<i>O. stellata</i>	91	0014	S.S. Turketti & S.S. Siqueira	Cape Agulhas	S 34 48.667'	E 20 03.338'	35.3	36.3	28.4	ns	1.15	1:1.4:2.1	17.6
<i>O. suteroides</i>	24	0026	S.S. Siqueira & J. Zietsman	Nieuwoudtville	S 31 20.277'	E 19 15.563'	30.2	32.1	37.7	ns	0.97	1:1.4:2.2	8.6
<i>O. suteroides</i>	69	0013	L.L. Dreyer, K.J. Esler, J. Zietsman & S.S. Siqueira	Nieuwoudtville	S 31 26.260'	E 19 08.619'	38.6	28.7	32.7	ns	1.50	1:1.4:2.0	8.8
<i>O. tenella</i>	17	0020	S.S. Siqueira & J. Zietsman	Citrusdal	S 32 18.481'	E 18 54.513'	26.4	37.3	36.4	ns	2.52	1:1.4:2.1	11.3
<i>O. tenella</i>	20	0023	S.S. Siqueira & J. Zietsman	Clanwilliam	S 32 05.519'	E 19 03.783'	33.0	38.0	29.0	ns	1.22	1:1.5:2.2	7.7
<i>O. tenuifolia</i>	45	0004	S.S. Siqueira	Stellenbosch	S 33 55.982'	E 18 52.378'	39.8	33.3	26.9	ns	2.74	1:1.4:2.2	10.7
<i>O. tenuifolia</i>	48	0005	S.S. Turketti & S.S. Siqueira	Malmesbury	S 33 30.036'	E 18 39.833'	39.8	31.1	29.1	ns	1.95	1:1.4:2.1	7.7
<i>O. tomentosa</i>	84	0001	J. Zietsman	Malmesbury	S 33 25.217'	E 18 23.281'	25.5	70.9	3.6	**	42.46	1:1.5:2.2	4.3

Identification	Pop no	Collection Number	Collector	Major locality ^a	Longitude	Latitude	% L	% M	% S	IE	G	Organ height ratios	RI
<i>O. tomentosa</i>	85	0012	J. Zietsman	Saron	S 33 11.305' E 18 59.060'	26.0	44.8	29.2	ns	5.59	1:1.5:2.4	8.8	
<i>O. tomentosa</i>	86	0003	Informal collector K.J. Esler	Hermon	S 33 25.607' E 19 02.762'	37.5	31.7	30.8	ns	0.93	1:1.4:2.3	14.8	
<i>O. truncatula</i>	1	0001	S.S.Siqueira	Caledon	S 34 07.019' E 19 50.471'	31.3	34.4	34.4	ns	0.13	1:1.5:2.2	6.9	
<i>O. versicolor</i>	78	0006	S.S. Siqueira, K. Oberlander & F. Roets	Stellenbosch	S 33 52.681' E 18 48.095'	33.3	36.0	30.7	ns	0.32	1:1.4:2.2	7.8	
<i>O. viscosa</i>	13	0014	S.S.Siqueira & J. Zietsman	Citrusdal	S 32 39.223' E 18 57.321'	36.1	31.9	31.9	ns	0.41	1:1.6:2.6	21.8	
<i>O. xantha</i>	44	0053	S.S.Siqueira & J. Zietsman	Clanwilliam	S 32 01.774' E 19 11.523'	28.3	34.9	36.8	ns	1.29	1:1.4:2.0	7.5	
<i>O. zeekoevleyensis</i>	9	0003	S.S. Turketti & S.S.Siqueira	Caledon	S 34 16.611' E 19 31.452'	44.0	44.0	12.0	**	24.34	1:1.6:2.4	4.8	

Notes: $P < 0.05$ (*), $P < 0.01$ (**), ns = not significant, as determined through G-statistics. Significance determined through χ -square distributions with 2 degrees of freedom. Lloyd's reciprocity index na = not applicable.

^a Additional information of any population and site such as minor and precise locality information, landscape, geology, soil moisture capacity, and habitat details such as substrate, surrounding environmental characteristics, disturbance, exposure, plant descriptions and herbarium samples can be obtained from the corresponding author.