

# The functional role of birds as pollinators in southern Cape fynbos.

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**UNIVERSITY OF  
KWAZULU-NATAL**

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## ABSTRACT

Nectarivorous birds, particularly sunbirds, are important pollinators of plants in the Cape Flora of South Africa, being responsible for pollinating approximately 5% of plant species. However, interactions between plants and nectarivorous birds in the eastern part of the Cape Floristic Region have not received much attention. This study focussed on two putatively bird-pollinated plant species found within the Nature's Valley area, namely *Kniphofia uvaria* and *Chasmanthe aethiopica*. The breeding systems were determined for each species and, due to their patchy population distribution, potential Allee effects on plant fecundity were also tested for. Flowering phenology in the area was also examined to test for associations between flowering of bird-pollinated plant species and the temporal presence of nectarivorous birds.

Selective exclusion experiments showed that sunbirds were the main pollinators of *K. uvaria* and *C. aethiopica* – fruit set and the number of viable seeds were much higher for untreated (open control) individuals where birds could visit flowers freely, compared to caged individuals which only allowed for insect visitation. Very few seeds developed when plants of the two species were bagged to exclude all pollinators, indicating that the species are not capable of autonomous self-fertilization.

Fruit and seed set were determined for patches of *K. uvaria* and *C. aethiopica* in order to test for potential Allee effects. There was a significant relation between the percentage of flowers that set fruit and the number of plants per patch for both *K. uvaria* and *C. aethiopica*. However, the number of seeds set per flower of *K. uvaria* and *C. aethiopica* had no significant relation with the number of plants per patch.

Flowering phenology for the area was determined by bi-weekly walks along the two study sites to document plants in flower. Bird presence was determined using data collected from bi-weekly mist netting sessions at the two sites. We then compared the presence of birds with the flowering data of bird-pollinated plant species. For the Kalandar Kloof site there were ten bird-pollinated plant species found and eight for the Salt River site. A comparison of the flowering data with bird presence data, indicated that nectar feeding birds were more likely to be present when accessible nectar availability is high. A total of 135 plant species across both sites were documented during the period of this study of which 14 were bird pollinated.

PREFACE

The data used in this thesis were collected in the Republic of South Africa from April 2014 to August 2016. All field observations were carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Dr Mark Brown and Professor Steven D. Johnson.

This thesis, submitted for the degree of Masters of Science in the College of Agriculture, Science and Engineering, University of KwaZulu-Natal, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of other, it is duly acknowledged in the text.

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Kellyn J. Whitehead

January 2018

I certify that the above statement is correct and as the candidate's supervisor I have approved this thesis for submission.

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Professor Steven D. Johnson

Supervisor

January 2018

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Dr Mark Brown

Co-supervisor

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DECLARATION 1 – PLAGIARISM

I, Kellyn J. Whitehead, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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DECLARATION 2 – PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

*Publication 1*

Evidence for bird-pollination in the African poker plant *Kniphofia uvaria* (Asphodelaceae).

Whitehead, K.J.; Brown, M. & Johnson, S.D. Evidence for bird-pollination in the African poker plant *Kniphofia uvaria*. In prep.

KJW conceived paper with MB and SDJ. KJW collected and analysed data, and wrote the paper.

MB and SDJ contributed comments to the manuscript.

*Publication 2*

Evidence for bird-pollination in the Cape geophyte *Chasmanthe aethiopica* (Iridaceae).

Whitehead, K.J.; Brown, M. & Johnson, S.D. Strong evidence for bird-pollination in the endemic irid *Chasmanthe aethiopica*. In prep.

KJW conceived paper with MB and SDJ. KJW collected and analysed data, and wrote the paper.

MB and SDJ contributed comments to the manuscript.

*Publication 3*

Tests for Allee effects in patches of *Kniphofia uvaria* and *Chasmanthe aethiopica*.

Whitehead, K.J.; Brown, M. & Johnson, S.D. A potential Allee effect among a patchily distributed population of *Kniphofia uvaria*. In prep.

KJW conceived paper with MB and SDJ. KJW collected and analysed data, and wrote the paper.

MB and SDJ contributed comments to the manuscript.

*Publication 4*

Flowering phenology and seasonal incidence of nectarivorous birds in fynbos of the southern Cape.

Whitehead, K.J.; Brown, M. & Johnson, S.D. A population of *Chasmanthe aethiopica* may exhibit an Allee effect. In prep.

KJW conceived paper with MB and SDJ. KJW collected and analysed data, and wrote the paper.

MB and SDJ contributed comments to the manuscript.

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January 2018

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Where do I begin? I initially had no plans to do a Master's degree after finishing off my Honours degree year, and instead landed up volunteering with an amazing NPO, the Nature's Valley Trust. While volunteering for them the director, Dr Mark Brown, approached me to see if I would be willing to take some of the research work I had started and turn it into a Masters. Although hesitant at first, I am glad and most grateful to have taking on the project, which although has had many challenges and setbacks, has been the most amazing experience. I would like to thank Dr Mark Brown for having started me on this journey and added support and encouragement throughout the process. I would also like to thank my co-supervisor, Professor Steven Johnson, for taking a shot on me and my project having not met me before. His support and input have been most appreciated. I would also like to let both my supervisors know how incredibly grateful I am for the funding they put together for me to be able to do this Masters, as without it I would not have been able too. I would also like to thank the University of KwaZulu-Natal firstly for accepting my Masters proposal, and second for the bursary I was awarded for my second year of study.

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On a final note, I would like to dedicate this Masters to my grandfather Norman Cassell. He has always showed a keen interest in my studies right from my first year of undergrad. He would love to hear all my stories and adventures about all the new things I had learnt and done. Although he was not here to see me complete this thesis, I know he was watching me from above smiling down and cheering me on.

## CONTENTS

ABSTRACT.....	2
PREFACE.....	3
DECLARATION 1 – PLAGIARISM .....	4
DECLARATION 2 – PUBLICATIONS.....	5
ACKNOWLEDGEMENTS.....	6
LIST OF FIGURES.....	10
LIST OF TABLES:.....	13
<b>Chapter 1 - Introduction.....</b>	<b>14</b>
Mutualisms, coevolution and plant-pollinator interactions .....	14
Flower rewards .....	19
Allee effects .....	21
Phenology .....	23
References .....	27
<b>Chapter 2 .....</b>	<b>45</b>
Evidence for bird-pollination in the African poker plant <i>Kniphofia uvaria</i> (Asphodelaceae).....	45
Abstract.....	45
Introduction .....	45
Materials and Methods.....	47
Results.....	51
Discussion.....	56
Conclusion.....	58
Acknowledgements.....	59
References .....	59
<b>Chapter 3 .....</b>	<b>63</b>
Evidence for bird-pollination in the Cape geophyte <i>Chasmanthe aethiopica</i> (Iridaceae).....	63
Abstract.....	63
Introduction .....	63
Material and methods .....	65
Results.....	68
Discussion.....	72
Conclusion.....	73
Acknowledgements.....	73
References .....	73
<b>Chapter 4 .....</b>	<b>76</b>

Tests for Allee effects in patches of <i>Kniphofia uvaria</i> and <i>Chasmanthe aethiopica</i> .....	76
Abstract.....	76
Introduction .....	76
Results.....	82
Discussion.....	88
Conclusion.....	90
Acknowledgements.....	90
References .....	90
<b>Chapter 5</b> .....	95
Flowering phenology and seasonal incidence of nectarivorous birds in fynbos of the southern Cape. ....	95
Abstract.....	95
Introduction .....	95
Materials and methods.....	97
Results.....	99
Discussion.....	105
Conclusion.....	107
Acknowledgements.....	107
References .....	107
Appendix 1 – Plant species list for Kalander Kloof for presence and absence. ....	112
Appendix 2 – Plant species list for Salt River for presence and absence.....	117
Appendix 3 – Bird species list for presence and absence. ....	122
<b>Chapter 6 - Conclusion</b> .....	126
Overview .....	126
Contribution to biological and ecological knowledge.....	126
Functional role of birds.....	126
Summary statement .....	127
Suggestions for future research.....	128
References .....	128

## LIST OF FIGURES

### Chapter 2:

- Figure 1: *Kniphofia uvaria* being visited by specialist nectarivore bird species (a) a male and (b) female Southern Double-collared sunbird. 48
- Figure 2: Exclusion experiments depicting (a) a bagged plant and (b) a caged plant. (c) shows a section of the study once bags and cages had been placed. 50
- Figure 3: Seeds of *K. uvaria*. The seed on the left is classified as non-viable and the seed on the right is viable. 51
- Figure 4: Visitation of insects to *K. uvaria*. A bee with pollen sacs (circled in red) on its legs approaching an inflorescence. 52
- Figure 5: The effect of pollinator exclusion on percentage fruit set in *K. uvaria*. Different letters show a significant difference. Box plot show medians. 54
- Figure 6: The difference in fruit set between typical (a) bagged, (b) caged and (c) open inflorescences. 54
- Figure 7: Difference between the bagged, caged and open treatments for the number of viable seeds produced in *K. uvaria*. Different letters show a significant difference. Box plots show medians. 55
- Figure 8: The difference between the bagged, caged and open treatments for the total number of seeds per fruit (viable and non-viable). Different letters show a significant difference. Box plots show medians. 55

### Chapter 3:

- Figure 1: A Southern Double-collared sunbird drinking nectar from a floral tube of *C. aethiopica*. Note position of anthers on birds' head, and subsequent possible pollen deposition with anthers touching head. 68
- Figure 2: The difference between the bagged, caged and open treatments for the percentage fruit set. Different letters show a significant difference. Box plots show medians. 70
- Figure 3: The difference in number of fruits and fruit size for the (a) bagged, (b) caged and (c) open inflorescences. 70

**Figure 4:** The difference between the bagged, caged and open treatments for (a) the number of viable seeds and (b) the total number of seeds (viable and non-viable). Different letters show a significant difference. Box plots show medians. 71

**Figure 5:** Difference in seed size for *C. aethiopica*. (a) seed size for an open treatment (first two seeds), caged treatment (third and fourth seeds) and bagged treatment (last seed). (b) seeds removed from the fruit of a caged plant and (c) fruits with seeds of a control (open) plant. 71

#### Chapter 4:

**Figure 1:** Population map for the year 2016 for *K. uvaria*. Shows the distribution of the patches (50 total) along the R 102 towards Nature's Valley. Patch sizes are marked with different sized circles. 80

**Figure 2:** Population map for the year 2016 for *C. aethiopica*. Shows the distribution of patches (27 total) throughout the residential area of Nature's Valley. Patch sizes are marked with different sized circles. 81

**Figure 3:** The relationship between patch size and (a) average number of fruits, (b) average percentage fruit set, (c) average number of viable seeds and (d) average total number of seeds (viable and non-viable) for *K. uvaria*. 83

**Figure 4:** The relationship between distance to nearest neighbour and (a) average number of fruits, (b) average percentage fruit set, (c) average number of viable seeds and (d) average total number of seeds (viable and non-viable) for *K. uvaria*. 84

**Figure 5:** The relationship between patch size and (a) average number of fruits, (b) average percentage fruit set, (c) average number of viable seeds and (d) average total number of seeds (viable and non-viable) for *C. aethiopica*. 86

**Figure 6:** The relationship between distance to nearest neighbour and (a) average number of fruits, (b) average percentage fruit set, (c) average number of viable seeds and (d) average total number of seeds (viable and non-viable) for *C. aethiopica*. 87

#### Chapter 5:

**Figure 1:** Flowering times for (a) non bird-pollinated and (b) bird-pollinated plant species. 101

**Figure 2:** The presence of Cape sugarbirds (black bars) and the number of protea species (grey bars) in flower for (a) 2014, May through to December, (b) 2015, January through to December and (c) 2016, January through to September. 102

Figure 3: The presence of (a) Orange-breasted sunbirds and (b) Southern Double-collared sunbirds for the period of May 2014 to September 2016. (c) shows the number of erica species in flower. Error bars show standard error. 104

Figure 4: The percentage recapture rate for the three main specialist bird species over the three-year period. CSB = Cape sugarbird, OBS = Orange-breasted sunbird and SDC = Southern Double-collared sunbird. Error bars show standard error. 105

## LIST OF TABLES:

### *Chapter 2:*

<u>Table 1</u> : Observations of birds visiting <i>K. uvaria</i> inflorescences.	52
<u>Table 2</u> : Bird visitation rates versus bee visitation rates of <i>K. uvaria</i> flowers.	53
<u>Table 3</u> : Floral and nectar mean measurements with standard errors.	53

### *Chapter 3:*

<u>Table 1</u> : Observations of birds visiting <i>C. aethiopica</i> inflorescences.	68
<u>Table 2</u> : Floral and nectar mean measurements with standard error.	69

### *Chapter 4:*

<u>Table 1</u> : Multiple regression models of the relationship between two predictor variables (patch size and distance to nearest neighbour) and measures of seed and fruit set for <i>K. uvaria</i> .	85
<u>Table 2</u> : Multiple regression models of the relationship between two predictor variables (patch size and distance to nearest neighbour) and measures of seed and fruit set for <i>C. aethiopica</i> .	88

### *Chapter 5:*

<u>Table 1</u> : The three families making up the most species found for Kalander Kloof.	99
<u>Table 2</u> : Bird-pollinated plant species along the Kalander Kloof hiking trail.	99
<u>Table 3</u> : The six families making up the most species found for Salt River.	100
<u>Table 4</u> : Bird-pollinated plant species along the Salt River hiking trail.	100

## **Chapter 1 - Introduction**

### **Mutualisms, coevolution and plant-pollinator interactions**

Angiosperms are the most diverse group within the plant kingdom (Ollerton, 1996) and the species within this group exhibit a wide diversity in the form of their flowers (Campbell *et al*, 1996; Mayfield *et al*, 2001). Barrett and Harder (1996) identified three features of plants that make their mating complicated. The first feature is that plants do not move like animals and therefore rely on outside vectors to transfer pollen. Secondly, a large majority of plants can self-pollinate, but this is at the potential expense of outcrossing. Finally, the male and female parts of the plants can come in many structural and temporal combinations. Pollination systems are known to be labile, even within species, and can evolve at a fast rate (Ollerton, 1996).

Flowering plants have over time evolved a range of characteristics that promote mating including variations in colour, scent, floral morphology and the rewards they may offer potential pollinators, such as nectar (Proctor *et al*, 1996; Mayfield *et al*, 2001).

Mutualistic relationships are believed to have formed over a long period of time and often begin as antagonistic interactions between the species involved (Pellmyr & Thompson, 1992). Boucher *et al* (1982) define mutualism as “an interaction between two species that is beneficial to both.” Mutualistic relationships can be beneficial in terms of nutritional; energy; protection and transportation benefits (Boucher *et al*, 1982). Although mutualisms are predominantly beneficial relationships, conflicts do occur causing a range of outcomes, from positive to negative (Anstett *et al*, 1997). Negative interactions between partners may impede progress and slow the evolution of the interaction down. Conflicts within mutualisms can have negative impacts which can lead to noticeable changes such as a decline in pollinator abundance (Rathcke, 2000); a change in resources available (Jennersten, 1988) and exclusion of true pollinators from flower resources by inefficient pollinators (Huryn, 1997). This may often be a result of changes in environmental conditions (Bronstein, 1994; Anstett *et al*, 1997).

An important mutualism which has gained a lot of attention is that between plants and their pollinators (Petanidou *et al*, 2008). Plant-pollinator interactions go back as far as the Cretaceous period, when foraging of insects on plants resulted in a substantial increase in plant reproductive success (Kearns & Inouye, 1997). Plant-animal

relationships are under strain due to anthropogenic activities (Bond, 1994; Potts *et al*, 2010) including: use of pesticides (Brittain *et al*, 2010; Brittain & Potts, 2011) which poison pollinators; modification and fragmentation of land and habitats (Steffan-Dewenter *et al*, 2002; Harris & Johnson, 2004), and the introduction of invasive species (Brown *et al*, 2002; Stout & Morales, 2009). These disruptions to plant-animal mutualisms could lead to a decrease in pollinators and change the density dynamics of plant and animal populations which are able to survive the disruption (Bond, 1994).

Mutualistic relationships are often misunderstood and presumed to be a coevolved relationship (Janzen, 1980), which is indeed the case in some instances, but not all. Janzen (1980) defined coevolution as “an evolutionary process where there is a change in a particular trait of the first population involved in response to a particular trait found in the second population, this then leads to a change of a particular trait in the second population in response to the change that occurred in the first”. Coevolution is a concept which has attracted much controversy in evolutionary biology with some suggesting it plays a significant part in shaping the biotic world (Thompson, 1989; Anderson & Johnson, 2008), while others suggest an overestimation in its role in the generation of species diversity (Boucher *et al*, 1982).

One of the first examples of a coevolutionary relationship being studied goes as far back as Darwin. Darwin (1862) hypothesized about the relationship between floral spur length of an orchid species and the proboscis of its moth pollinator. He suggested that long-spurred flowers evolved to accommodate a long-tongued pollinator and vice versa, which over time created a more specialized relationship between the flower and its pollinator. Since Darwin’s suggestion there has been a substantial increase in the amount of attention given to plant-pollinator interactions. Studies on specialized plant-pollinator systems include those involving moths (Alexandersson & Johnson, 2002; Goldblatt & Manning, 2002), birds (Campbell *et al*, 1996; Muchhala, 2003; Geerts & Pauw, 2009), butterflies (Cruden & Hermann-Parker, 1979; Goldblatt & Manning, 2002), other insects (Goldblatt *et al*, 1995; Johnson & Steiner, 1997; Goldblatt & Manning, 2000; Hargreaves *et al*, 2008), reptiles (Olsen & Valido, 2003) and mammals (Wiens & Rourke, 1978; Muchhala, 2003).

The coevolution of plant-pollinator interactions is believed to have been the primary reason for the pollination syndromes we see today (Gilbert & Raven, 1975; Janzen, 1980;

Sakai *et al*, 1999). The morphology of the plants in these interactions play an important role with regards to the placement of pollen and pollen acquirement by the stigma from the pollinator (Sakai *et al*, 1999). Floral traits not only function to attract pollinators, but also encourage them to forage in a way such that there is a high rate of compatible pollen transfer, i.e. to keep pollinators “faithful” (Campbell, 1985; Wolfe & Sowell, 2006). Another factor to consider when looking at animal pollination in plants is the quality and quantity at which they collect and deposit pollen and the efficiency of the pollinator (Schemske & Horvitz, 1984; Herrera, 1987; Olsen, 1997; Mayfield *et al*, 2001). This efficiency is referred to as the “most effective pollinator principle” and was coined by Stebbins (Stebbins, 1970; Mayfield *et al*, 2001). Stebbins (1970) suggested that the efficiency of a pollinator will be positively related throughout different kinds of visitors to the flowers of a particular plant species, and that the characteristic which promotes this efficiency would be spread through the population as a result of natural selection.

Flowers which are pollinated by animals (zoophilous) are designed in a specific way; pollen is placed on certain parts of the pollinator’s body in a way that is most efficient for accurate pollen placement on the stigma (Proctor *et al*, 1996; Cresswell, 1998). Ollerton (1996) highlighted four themes with regards to plant-pollinator interactions, the first being that many angiosperms are generalists, secondly there is a large range of pollinators during a flowering period, thirdly, a variation in main pollinators can occur between seasons and finally, the characteristics of the flowers can determine the type of pollinator. There are a very large number of plant-pollinator interactions that have been documented (Feinsinger *et al*, 1986; Johnson, 1996; Alexandersson & Johnson, 2002; Johnson, 2004; Anderson & Johnson, 2008; Hargreaves *et al*, 2008; Brown *et al*, 2009 and references therein) and the plants involved in these interactions usually have a certain set of characteristics to attract and conform morphologically to the pollinator (van der Pijl, 1961). These are referred to in the literature as pollination syndromes and these syndromes refer to a pattern of convergent evolution among plant species that adapt to the sensory systems and morphology of particular pollinators (Mayfield *et al*, 2001). Pollination syndromes have been questioned in the literature with regards to how reliable they are and whether they are as specialized as previously believed (Waser *et al*, 1996; Ollerton, 1998; Hingston & McQuillan, 2000; Johnson & Steiner, 2000; Hargreaves *et al*, 2004; Johnson & Wester, 2017). Studies have shown that some seemingly specialized pollination systems are more generalized than previously thought (Waser *et*

*al*, 1996; Hargreaves *et al*, 2004). Furthermore, the importance of a certain pollinator does not depend only on visitation rate but by how successfully they are able to transfer pollen (Carthew, 1993; Hargreaves *et al*, 2004) and how much pollen they carry (Coetzee & Giliomee, 1985; Hargreaves *et al*, 2004).

Pollination syndromes include: beetle-pollination (cantharophily) - flowers have pollen or food body traps, no significant shape, open nectar (nectar not hidden), lack nectar-guides and have a robust odour (fruity or aminoid) (van der Pijl, 1961; Hingston & McQuillan, 2000); bee-pollination (melittophily) - flowers have small amounts of hidden nectar, a nectar-guide, not red in colour, an alighting (resting place) and have zygomorphic (bilateral) symmetry (van der Pijl, 1961; Hingston & McQuillan, 2000); Hawkmoth-pollination (sphingophily) - flowers tend to have an odour which is sweet in scent, white/green colour, a narrow floral tube or have a spur, no resting place, anthers which are exposed at night (nocturnal anthesis) and can move, lots of nectar and a deep dissection (van der Pijl, 1961; Hingston & McQuillan, 2000); butterfly-pollination (psychophily) - flowers come in a range of different colours, have a narrow floral tube or spur, a slight odour, soft tissues and the anthers are exposed during the day (diurnal anthesis) (van der Pijl, 1961; Hingston & McQuillan, 2000); bat-pollination (chiropterphily) - flowers are usually white or dull in colours, large flowers or inflorescences, a substantial amount of nectar, unpleasant odour, with exposed anthers at night (van der Pijl, 1961). Other forms of pollination which have been studied include rodent pollination (Johnson *et al*, 2001; Kleizen *et al*, 2008; Wester *et al*, 2009) and reptile pollination (Olsen & Valido, 2003).

Bird-pollination (ornithophily) has received increased attention since the early 1980s (Linhart & Feinsinger, 1980; Collins, 1983; Rebelo *et al*, 1984; Rebelo, 1987; Wolf & Stiles, 1989; Johnson, 1996; Pauw, 1998; Johnson & Brown, 2004; Wester & Claßen-Bockhoff, 2006; Brown *et al*, 2009; Geerts & Pauw, 2009; Brown *et al*, 2010). Characteristics of flowers which are pollinated by birds include vivid colours (usually red), no nectar-guide, no smell, large volume of nectar deep within the corolla tube, tubular corollas or “brush” inflorescence, fusion of floral petals to provide strength, a capillary system for nectar retrieval, and diurnal anthesis (van der Pijl, 1961; Brown & Kodric-Brown, 1979; Faegri & van der Pijl, 1979; Johnson, 1996; Hingston & McQuillan, 2000; Johnson & Nicolson, 2008; Geerts & Pauw, 2009).

Generalized pollination systems may result in weaker selection on floral traits (Herrera, 1988; Johnson & Steiner, 2000) whereas specialized systems may result in stronger selection for particular floral traits (Nilsson, 1988; Galen, 1996; Johnson & Steiner, 2000). For example, in a specialized system you may find that the plant has developed a trait which deposits pollen onto a specific part of its bird pollinator (See Pauw, 1998; Johnson & Brown, 2004) whereas in a generalist system the pollen would be placed on no specific part of the pollinator. Although many sunbird and sugarbird species show a specialization with the plant species they pollinate (Geerts & Pauw, 2009; Geerts & Pauw, 2012) there are a few exceptions that show some generalist bird species, which are opportunistic nectarivores, pollinate flowers which are also specialized in terms of floral traits (Johnson *et al*, 2006; Brown *et al*, 2009).

There is a large amount of studies that can be found in the literature which look at specialized versus generalized plant-pollinator systems (Gomez & Zamara, 1999; Bascompte *et al*, 2003; Vazquez & Aizen, 2006; Waser & Ollerton, 2006; Stang *et al*, 2007; Petanidou *et al*, 2008). Until recently, it has been assumed that specialization within plant-pollinator relationships is symmetric, with specialist plants interacting with specialist pollinators and generalist with generalists (Schemske, 1983; Vazquez & Simberloff, 2002; Vazquez & Aizen, 2004). It is becoming more evident that, at a community level, specialization is more asymmetric with specialized plants having generalist pollinators and a specialist pollinator using generalized plants. (Vazquez & Simberloff, 2002; Bascompte *et al*, 2003; Vazquez & Aizen, 2004; Basilio *et al*, 2006; Petanidou & Potts, 2006; Vazquez & Aizen, 2006; Petanidou *et al*, 2008). When a plant uses a pollination system where the primary pollinators are visitors whose movements are unpredictable, they may include a number of fail-safe adaptations to increase the chance of reproduction (Wolf & Stiles, 1989). These adaptations could include the ability to self-pollinate (Stebbins, 1970; Paige & Whitham, 1978; Rathcke, 1988) and having floral traits which attract many pollinators (Pleasants & Waser, 1985; Rathcke, 1988).

## **Flower rewards**

One way plants attract suitable pollinators is by producing floral rewards for their pollinators. The type of reward on offer often influences what type of pollinator will be attracted to the flower as well as how accessible that reward is to that pollinator (Armbuster & Muchhala, 2009). These rewards include nectar and pollen (Stang *et al* 2009), oils (Wright & Schiestl, 2009), fragrances, waxes and resins (Ackerman *et al*, 1994). Of these, nectar is the most common reward used by flowering plants (Stiles & Freeman, 1993; Ornelas *et al* 2007). The production of nectar comes at a high-energy cost in terms of both producing the nectar, and making the structures required to produce the nectar (Nicolson & Fleming, 2003), leading to a reproductive cost for the plant (Southwick, 1984; Pyke, 1991; Ornelas *et al*, 2007). The structures used to produce nectar are called nectaries and can be found either on the surface, embedded deeply or be a protrusion from the organ which holds them (Pacini *et al*, 2003).

Baker (1977) looked at the non-sugar constituents found within nectar and identified four classes namely amino acids, lipids, antioxidants and toxic substances. Amino acids make up the highest concentration within the nectar and is what provides the pollinator with a source of protein (Baker, 1977). There are three main sugar components found in nectar which are the disaccharide sucrose and the monosaccharides glucose and fructose (Percival, 1961; Stiles & Freeman, 1993; Rusterholz & Erhardt, 1997; Baker *et al*, 1998; Lotz & Schondube, 2006). The sugar concentration in nectar can vary between plant species from anywhere between five percent to sixty-six percent (Schondube & Martinez del Rio, 2003) with the type of sugar present in abundance varying from hexose (glucose and fructose) rich to sucrose rich nectars (Schondube & Martinez del Rio, 2003). Some nectars contain unpalatable secondary compounds such as alkaloids and phenolics (Johnson *et al*, 2006) which could act as deterrents or filters for plants to prevent unwanted visitors (Stephenson, 1981, 1982).

The amount of resources available changes with time and can influence the way bird communities are structured (Symes *et al*, 2007). Birds which feed on nectar respond to the amount of nectar available, and several studies have shown an increase in the number of birds in response to an increase in nectar availability (Brown & Hopkins, 1996; Franklin & Noske, 1999; Cotton, 2006). In southern Africa, sunbirds and sugarbirds also respond to nectar resources and have been recorded making use of nectar which is

seasonally available (Tree, 1990; Craig & Simon, 1991; de Swardt, 1991; Symes *et al*, 2001). The success of pollination for a plant is determined by how well the pollinator pollinates, and nectar can have an influence on this (Cresswell, 1999). First, the presence of a reward encourages the pollinator to visit the plant (Faegri & van der Pijl, 1979) and the amount available may influence the time spent foraging (Real & Rathcke, 1991). Second, the amount of nectar found within a flower may determine whether the pollinator will explore more flowers on the plant (Pyke, 1978; Galen & Plowright, 1985; Cresswell, 1990; Cresswell, 1999), which in turn could affect self-pollination (de Jong *et al*, 1993; Harder & Barrett, 1996). Finally, the amount of nectar available in a single flower could potentially influence how the pollinator removes the nectar, and how efficient pollen transfer among flowers will be (Thomson & Plowright, 1980; Cresswell, 1999).

A study by Johnson & Nicolson (2008) looked at the association between properties of nectar and specificity in bird-pollination systems. They developed a database containing information of the nectar properties of bird-pollinated plant species for Africa and the Americas. Significant differences were found for nectar properties between plants adapted for specialist nectar feeding birds and those adapted for opportunistic nectarivorous birds (Johnson & Nicolson, 2008). Sunbird adapted flowers had nectar volumes of 10 – 30  $\mu$ l, 15 – 25% concentration and sucrose content of 40 – 60%. Opportunistic bird-pollinated flowers had nectar volumes of 40 – 100  $\mu$ l, 8 – 12% concentration and 0 – 5% sucrose content.

The production of nectar can have both positive and negative effects for the plant (Bradenburg *et al*, 2009). The positives of nectar include a potential increase in pollinator visits (Pleasants, 1981; Real & Rathcke, 1991; Leiss & Klinkhamer, 2005); bout length between visits (Mitchell, 1993, Gonzalez *et al*, 1995; Leiss & Klinkhamer, 2005) and the amount of time a pollinator spends at a flower (Galen & Plowright, 1985; Cresswell, 1999). Negatives of having nectar include the cost of producing the nectar (Southwick, 1984; Pyke, 1991; Bradenburg *et al*, 2009); attracting unwanted visitors such as nectar robbers and certain microbes which consume the nectar without pollinating (Bradenburg *et al*, 2009) and the potential of pollen of one species being transferred to another species which also has nectar as a reward (Bradenburg *et al*, 2009).

## **Allee effects**

Plant populations are under increasing threat due to invasive species (Ghazoul, 2004), habitat fragmentation due to habitat loss and urbanisation (Lundberg & Moberg, 2003; Johnson, 2004; Pauw & Louw, 2012), climate change (Hannah *et al*, 2002; Dawson *et al*, 2011; Wise *et al*, 2012) and land alteration (Gess & Gess, 1993; Kearns & Inouye, 1997). As a result, many plant populations are decreasing in size and becoming fragmented or 'patchy'. Plants may also occur naturally in patches ranging from a few to many thousands of plants. When small patches show reduced fecundity of individuals or reduced demographic viability, this is known as the Allee effect (essentially a form of "underpopulation"). The Allee effect is a concept which has been met with confusion, pertaining to both its meaning and due to misuse in the literature (Stephens *et al*, 1999). The concept refers to the problem of finding potential mates with low population densities (Myers *et al*, 1995; Amarasekare, 1998); a drop in fitness with small population size (McCarthy, 1997; Fischer & Matthies, 1998) and has been used to define negative density dependence (Levitan *et al*, 1992).

In essence, the Allee effect is a decrease in a population's growth rate with regards to low size or a reduction in fecundity that may contribute to reduced population growth (Allee *et al*, 1949; Deredec & Courchamp, 2007; Cappuccino, 2004). The benefits of having a large population size was outlined by Stephens *et al* (1999) and include predator repletion; social thermoregulation; increased chance of pollination; a decrease in inbreeding and a higher success for fertilization. The potential causes of Allee effects could be genetic, demographic or ecological (Forsyth, 2003) and include extinction (Dennis, 2002; Boukal & Berec, 2002), establishment (Drake & Lodge, 2006; Liebhold & Tobin, 2006), metapopulation variations (Zhou *et al*, 2004; Martcheva & Bolker, 2007), predator-prey interactions (Gascoigne & Lipcius, 2004; Morozov *et al*, 2004) and the spread of parasites (Deredec & Courchamp, 2006).

Smaller populations are more likely to have lower reproductive success compared to larger populations as there are less potential mates or fewer potential pollinators, which may then lead to a greater chance of extinction (Stephens *et al*, 1999; Bossuyt, 2007; Levin *et al*, 2009). There are two levels of which Allee effects can be studied, namely component and demographic. The former refers to the relationship between the fecundity of an individual and the number of conspecifics within a population (Stephens

*et al*, 1999; Deredec & Courchamp, 2007; Gregory *et al*, 2010) and the latter refers to the relationship between the number of individuals in the population and the rate at which the population grows (Stephens *et al*, 1999; Deredec & Courchamp, 2007; Gregory *et al* 2010). There are several examples where the Allee effect is evident and this evidence comes from species with patchy occupancy patterns and fragmented habitats (Amarasekare, 1998). Lamont *et al* (1993) looked at small populations of *Banksia goodii* and found that approximately half the population did not produce any seeds due to limited pollinator presence caused by small population size. Thiollay & Meyburg (1988) did a study on the Javan hawk eagle (*Spizaetus bartelsi*) and found that this species has few to no opportunities to colonize as they are restricted to three forest reserves. Thomas & Hanski (1996) found that the British butterflies *Hesperia comma* and *Plebjus argus* are rarely seen, or absent, in areas with less than ten patches of their required habitat, southerly-facing hillside grassland.

Plants which, in many circumstances, need an animal as a vector for pollination can be at risk for Allee effects which might result due to low density, patches of a small size or patches which are isolated (Groom, 1998). The size of a patch or population of plants could influence the reproductive success of a species more directly (Groom, 1998) than any of the other mentioned possible causes. The reason for this being that pollinators may be less attracted to smaller displays of flowers which will then lead to fewer visits to flowers, and affect the quality and quantity of pollination services (Kunin, 1993; Ingvarsson & Lundberg, 1995; Groom, 1998; Hackney & McGraw, 2001). Some studies show the negative effect pollinator limitation has on small populations of plants (Kunin, 1997; Forsyth, 2003; Kéry & Matthies, 2004; Waites & Agren, 2004; Steven & Waller, 2007), although there are examples where no effect is found (Wilson *et al*, 2009), suggesting some pollinators are still attracted to small patches.

Jennersten (1988) looked at the effects habitat fragmentation was having on a butterfly-pollinated, caryophyllaceous herb, *Dianthus deltoides*. It was found that the highly fragmented site had an overall lower diversity and abundance of flowering plants as well as flower-visiting insects (Jennersten, 1988). Thus, the flowers in the fragmented area had fewer visits and a lower seed set (Jennersten, 1988). Aizen and Feinsinger (1994) did a comparison of small forest fragments to larger, more continuous, forest patches. Within each patch they compared pollination levels, fruit and seed set (Aizen & Feinsinger,

1994). They found that the smaller patches of forest produced a lower pollination level, fruit set and seed set compared to the larger forest patches (Aizen & Feinsinger, 1994). Kunin (1993) looked at the effect of population density on pollinator visitation for wild mustard (*Brassica kaber*). He found that pollinator visits to flowers decreased sharply when population density was low, and that widely spaced plants had a much lower seed-set (Kunin, 1993). A study by Hackney & McGraw (2001) looked at the effect of population size on the reproductive success of American ginseng (*Panax quinquefolius*). Budding, flowering and green and mature fruit was recorded for the flowering season in summer (Hackney & McGraw, 2001). They found that the production of fruit per plant increased as flowering population size increased, suggesting a possible Allee effect for small populations (Hackney & McGraw, 2001). A study by Ward and Johnson (2005) looked at seed production and recruitment of juveniles in *Brunsvigia radulosa*. They correlated this with habitat fragmentation, population size and population isolation (Ward & Johnson, 2005). Seed production was found to have a significant positive relationship with population size, with reduced seed production in smaller population sizes compared to larger ones (Ward & Johnson, 2005). It was found that this was a result of pollen limitation which had an effect on demographics, with less juvenile recruitment in small populations (Ward & Johnson, 2005). Johnson *et al* (2004) studied the endangered daisy *Gerbera auraniaca* and various environmental factors on seed production and seedling recruitment. They found that smaller populations produced a significantly lower mean number of seeds compared to larger populations, with pollen limitation the reason (Johnson *et al*, 2004). In short, there is ample evidence to suggest that reduced population size adversely affects fecundity in plants that depend on animal pollinators.

### **Phenology**

Phenology is the study of the timing at which plants or animals undergo biological changes, for example, flowering, moulting, breeding and growth (Lieth, 1974). Another term which is used in phenology studies is 'phenophase' which refers to a particular stage during the development of a plant, which is restricted to a certain time frame (Monasterio & Sarmiento, 1967). Studying the phenology of an area, ecosystem, species, or biome helps determine if factors such as climate change (Cleland *et al*, 2007; Gordo & Sanz, 2010; Korner & Basler, 2010; Richardson *et al*, 2013) and habitat fragmentation (Neil &

Wu, 2006; Dubois & Cheptou, 2017) are having an impact on our ecosystems. Several factors could lead to changes in phenology such as resources changing, temperature and photoperiod changes (Way & Montgomery, 2015; Lange *et al*, 2016), with others including soil (Zelikova *et al*, 2015), moisture and humidity (Pierce, 1984).

Within a plant community, species compete for resources (water, nutrients, pollinators, seed dispersers, etc.) needed for growth, seed dispersal and pollination (Pierce, 1984). Resource partitioning within the community allows for less competition between species, allowing co-existence, leading to higher diversity within that ecosystem (Pierce, 1984). One way in which plant communities can partition the required resources, pollinators and seed dispersers is to stagger the time of their phenophases (Pierce, 1984) by flowering at different times and offering rewards at different times. For example, Thomson *et al* (2000) did a study looking at a bee-pollinated *Penstemon* and bird-pollinated *Keckiella*. They found that the bee-pollinated plants had anthers which opened more gradually and not completely, whereas the bird-pollinated plants opened their anthers quickly and completely (Thomson *et al*, 2000). A study by Reader (1977) looked at two peat bogs which had the same three ericad species growing on them. They found that one of the reasons that these three species had successful pollination by the same bee species, was because they flowered at different times, therefore exposing their rewards of nectar at different times (Reader, 1977).

### **Study region**

South Africa is a country which is diverse in both fauna and flora and has a wide range of biomes from forest to savanna, grassland to desert. Of the biomes found in South Africa, the Fynbos is the most uniquely diverse, and is considered as one of the six floral kingdoms of the world (Good, 1974; Takhtajan, 1986; Goldblatt & Manning, 2002). Fynbos is endemic to South Africa and is found only within the Western and Eastern Cape provinces. The area covered in this amazing flora is approximately 90 000 km<sup>2</sup> (Goldblatt, 1978; Goldblatt & Manning, 2002) with an estimated number of species reaching over 9 030 species of which approximately 8 900 of those species are flowering plants (Goldblatt & Manning, 2000; Goldblatt & Manning, 2002), with many of these species being endemic to the fynbos (around 69%). Given the size of the biome and the number of species found within such a small area, the fynbos is one of the most diverse floral kingdoms next to tropical forest.

The southern Cape of South Africa is an area which contains amazingly diverse sections of fynbos which have been understudied compared to other areas of the Western Cape, such as Cape Town and Stellenbosch. The Garden Route, which covers an area starting from Riversdale to Storms River, is one of these areas that has had little attention. The Garden Route National Park falls within this area and is broken up into three sections (areas) namely the Wilderness, Knysna and Tsitsikamma sections. This study was done within the Tsitsikamma section of the Garden Route National Park just before the border between the Western and Eastern Cape. A few pollination studies which have occurred in the Garden Route include: a study by Steiner (1998) looking at beetle pollination in *Ceratandra grandiflora* in Plettenberg Bay, Nature's Valley and Witelbos; a study by Midgley (1989) looking at pollen dispersal of *Podocarpus falcatus* in the Knysna forest; and finally, a study by Johnson and Brown (2004) looking at a new pollination system in *Disa chrysostachya* which included a population in Coldstream.

### **Study species**

This study focussed on two putatively bird-pollinated plants - *Kniphofia uvaria* (Asphodelaceae) and *Chasmanthe aethiopica* (Iridaceae) - which grow in natural fynbos vegetation around Natures Valley. A literature search yielded only two results for pollination system studies of *K. uvaria* (Johnson, 1994; Newman *et al*, 2012) and only one paper broadly investigating the biology of all three species of *Chasmanthe* (Goldblatt *et al*, 2004). Geerts and Pauw (2009) used *C. aethiopica* as one of their study species as did Geerts *et al* (2012), with both studies looking at bird-pollination. The aforementioned studies of *K. uvaria* focused on utilization of the flowers as a nectar resource for butterflies and did not investigate the role of birds as pollinators of the species. The previous studies of *Chasmanthe* were limited to observations and did not include experimental approaches. In general, there are fewer studies done on plant pollination systems in the southern Cape compared to the south-western Cape region (some examples: Midgley, 1989; Steiner, 1998; Johnson & Brown, 2004; Manning & Goldblatt, 2005). For this reason, and the high likelihood of these two species being bird-pollinated and a potential food source for the bird community in the area, I initiated a study of these species in the De Vasselot region of the Tsitsikamma section of the Garden Route National Park. I studied the pollination biology of these two species using exclusion experiments

and by collecting floral and nectar data to compare with data for known bird-pollinated species.

Both species appeared to have a patchy distribution in their area of occurrence (*K. uvaria* along the road verges of the R102 connecting the N2 with Nature's Valley; *C. aethiopica* throughout Nature's Valley until the Salt River mouth). I therefore investigated potential Allee effects in the populations of these two species. I obtained data for different patches during the flowering seasons (June – August for *K. uvaria*; May – July for *C. aethiopica*) over multiple years. I collected fruit set and seed set data from each patch and compared between patches to see if small patches were less successful when compared to larger populations with regards to pollination success.

The fynbos of the southern Cape differs from that which is found in Cape Town and surrounds, such as Stellenbosch, as the climate is not the same. A study by Johnson (1992) looked at what climatic and phylogenetic characteristics determined flowering seasonality within the fynbos. It was found that there was a difference in flowering along a west (winter rainfall) to east (non-seasonal rainfall) gradient (Johnson, 1992). The fynbos to the west has a peak flowering time during spring with the fynbos to the east having a peak flowering time during early summer (Johnson, 1992). A review of the literature found several studies done in southern Cape fynbos, as a whole (Warren *et al*, 2011; Cowling *et al*, 2017) as well as for individual genera (Thuller *et al*, 2004). I documented species presence in the area to determine which of those provide a resource for the specialist nectar feeding bird population within the national park. We documented the species found along two sections of the national park (Kalandar Kloof and Salt River trails), identified them and recorded the months we found them flowering. We then compared the flowering times of the bird-pollinated species with the presence of sunbirds and sugarbirds from ringing data we had collected in both areas.

## **Hypotheses**

The main hypotheses in this thesis are as follows:

1. *Kniphofia uvaria* and *Chasmanthe aethiopica* will be reliant on bird-pollination for successful fertilization, with insects playing a small role in pollination.
2. The patchy distribution of the *Kniphofia uvaria* and *Chasmanthe aethiopica* populations will potentially result in an Allee effect among patches.
3. The presence of nectarivorous bird species will be correlated with flowering times of the species they feed from and pollinate.

The goal of this thesis is to take the data collected from each aspect of the study and determine the community context of the bird pollination systems of two focal plant species in the Tsitsikamma section of the Garden Route National Park. This thesis uses an experimental approach using exclusion experiments to determine pollination systems, where past studies have predominantly relied on observational data. With the addition of phenology data, I further aimed to determine whether there is a sufficient amount of resource availability for the bird community, and if there are periods where birds need to move to other areas, what can be done to conserve those areas to help protect not only the birds but also the vegetation.

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## **Chapter 2**

# Evidence for bird-pollination in the African poker plant *Kniphofia uvaria* (Asphodelaceae)

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### **Abstract**

As not all animal visitors to flowers are effective pollinators, the ecological reliance of plants on particular pollinators needs to be established by means of experimentation. We studied the pollination system of *Kniphofia uvaria*, a red-hot poker (Asphodelaceae), to determine whether nectar traits conform to the floral systems of bird pollination and to establish the main pollinator(s) of this species. Nectar traits were found to be similar to those of specialist bird-pollinated plant species. Few seeds were produced when all flower visitors were excluded, indicating that *K. uvaria* may not be capable of autogamy. Selective exclusion of birds led to a significant decrease in seed production relative to open-pollination, indicating that birds are key pollinators of this species. We conclude that *K. uvaria* is adapted to, and reliant on, birds for pollination.

*Key words: fruit set, Kniphofia uvaria, nectar, pollination syndrome*

### **Introduction**

Pollination is critical for reproduction of almost all flowering plants (Kearns *et al*, 1998; Johnson, 2004). Flowers exhibit convergent suites of adaptations (known as floral or pollination syndromes) in relation to particular animal groups. These pollination syndromes include flowers that are bird-adapted (ornithophily), bat-adapted (chiropterophily), and various insect-adapted (entomophily) (van der Pijl, 1961). Floral traits that are important components of pollination syndromes include scent, colour, size and shape (van der Pijl, 1961; Hargreaves *et al*, 2004).

While pollination syndromes can be investigated by studying evolutionary convergence in floral traits, verification that flowers are pollinated by particular animal groups (i.e. the "pollination system") requires that experimental work be done in an ecological context.

Pollination systems that have received much attention include those involving hummingbirds (Waser, 1978; Schemske, 1980; Altshuler, 2003), sunbirds (Frost & Frost, 1981; Geerts & Pauw, 2009), bees (Galen & Stanton, 1989; Johnson & Steiner, 1994; Pauw, 2006), butterflies (Cruden & Hermann-Parker, 1979; Johnson & Bond, 1994; Rusterholz & Erhardt, 1997), wasps (Shuttleworth & Johnson, 2006; Shuttleworth & Johnson, 2009) and moths (Fleming & Holland, 1998; Goldblatt & Manning, 2002).

It has been estimated that between 2.5 % to 4 % of plants species within South Africa are pollinated by birds (Johnson, 2004; Geerts & Pauw, 2009). Plants which are pollinated by birds generally conform to a floral syndrome that includes bright colours (mostly red and orange), large volumes of dilute nectar, a tubular shape, diurnal anthesis, the fusion of the petals and sepals to create strengthening of flowers and a lack of scent (van der Pijl, 1961; Hingston & McQuillan, 2000). The large volume of nectar and tubular shape of the flowers make them susceptible to nectar robbing (Irwin & Maloof, 2002), where birds and insects alike remove nectar from the flower by making holes or slits in the tube (Irwin & Brody, 1998). When comparing plants pollinated by specialist avian nectarivores with those pollinated by opportunistic avian nectarivores, there are some floral and nectar characteristics that differ. For example, a study by Johnson and Nicolson (2008), found that specialist bird-pollinated plants (e.g. sunbird or hummingbird pollination) have smaller nectar volumes, with high nectar concentrations and higher sucrose content, compared to opportunistic nectarivore bird-pollinated plants (e.g. Cape white-eyes or weaver pollination). Specialist bird-pollinated plants also tend to be tubular in shape whereas generalist bird-pollinated plants are more likely to have open flowers (non-fused petals) (Johnson, 2004).

The genus *Kniphofia* belongs to the family Asphodelaceae and are more commonly known as the red-hot poker (Dold & McMaster, 2005; Ramdhani *et al*, 2006). There are approximately 70 species known of which 47 are found within South Africa (Dold & McMaster, 2005) and many are rare with around 25 known species featuring on the red data list (Dold & McMaster, 2005). *Kniphofia* is found in Africa, southern Arabia and New Zealand (Manning, 2007) with the majority of species found within the winter rainfall and eastern areas of South Africa (Manning, 2007). Although some work has been done on the pollination syndromes of *Kniphofia* (Brown *et al*, 2009; Brown *et al*, 2010; Brown *et al*, 2011), little is known about the species *Kniphofia uvaria*, especially its pollination

biology. Johnson (1994) and Newman *et al* (2012) found that mountain pride butterflies *Aeroptes tulbaghia* use the flowers of this species as a source of nectar, but also noted visits to the flowers by sunbirds. Some studies on *Kniphofia* have found that there are species which are genetically self-incompatible including *Kniphofia caulescens* (Brown *et al*, 2009) and *Kniphofia laxiflora* (Brown *et al*, 2010), indicating that they are reliant on birds or insects for successful pollination.

The purpose of this study was to determine the pollination biology of *K. uvaria* by using pollinator exclusion experiments, visitor observations and to compare floral and nectar characteristics to the established syndromes of bird pollination (Johnson & Nicolson, 2008). We predicted that (a) *K. uvaria* would be dependent on birds for successful pollination, (b) would not be capable of self-pollination in the absence of pollinators and (c) that the nectar characteristics of *K. uvaria* would be similar to, or match, those of known bird pollinated plants.

## **Materials and Methods**

### Study site

This study was conducted in fynbos vegetation along the R102 (33° 57' 57.33" S; 23° 32' 36.05" E) towards Nature's Valley (33° 58' 50" S; 23° 33' 33" E). This patch of fynbos falls within the Tsitsikamma section of the Garden Route National Park. All data collection took place during the flowering period of 2016.

### Plant species

*Kniphofia uvaria* ("red-hot poker") belongs to the family Asphodelaceae and flowers from April through to August in the study region. *K. uvaria* grows to a height of approximately 50 cm to 120 cm and is usually found in small clumps. It produces green-yellow and orange flowers (Fig. 1) in a dense spike inflorescence (Manning, 2007).



**Figure 1:** *Kniphofia uvaria* being visited by specialist nectarivore bird species (a) a male and (b) female Southern Double-collared sunbird.

### Pollinator observations

Pollinator observations were done to determine what birds and insects were visiting *K. uvaria* and of these visitors, which were pollinating the flowers. A visitor was noted down as pollinating a flower if, when probing or crawling into a flower, contact was made with the reproductive parts of the flower. Pollinator observations were done over a two-day period, for two hours in the morning (08:30am – 10:30am). Plant-pollinator interactions were observed approximately 20m away from visiting birds (binoculars were used to observe the birds and their behaviour from this distance) and 1m away for visiting insects. The number of times a bird or insect probed a flower was recorded, along with time spent on an inflorescence, before moving to the next plant. A patch of between 10 and 15 plants was observed each time of bird observations. For insect observations, we sat in the middle of a patch of between 5 and 10 plants. Where possible photo evidence of visitation was taken.

### Floral and nectar measurements

Floral measurements were taken (to link floral and bird morphology) from flowers which were removed for nectar measurement purposes. Corolla length and width were measured, with digital callipers (Grip 150mm digital Vernier Calliper) to determine floral characteristics. Length was measured from the base of the ovary to the tip of the tube, as

this gives the distance from the lowest nectar level to the anthers and stigma and width at the widest part of the flower (floral aperture). The dimensions gathered were compared to those of known bird-pollinated plant systems.

Additionally, we determined standing crop nectar volume and twenty-four-hour nectar production. A five microliter ( $\mu\text{l}$ ) capillary tube was placed into the nectar at the base of the floral tube to measure standing crop nectar volumes. The volume of nectar drawn up by capillary action was recorded and the sucrose concentration determined using a refractometer (ATAGO). This was done for three flowers on each of 19 individual plant (N=57).

Twenty-four-hour nectar production was measured by removing nectar from the base of the flower using a 1-ml insulin syringe. This was done for each of three flowers on 19 individual plants (N=57). Emptied flowers were marked by tying string to the flower. After nectar extraction, the plant was bagged for a twenty-four-hour period to prevent visitation from potential pollinators. After 24 hours the bag was removed and nectar recorded from marked flower using the capillary method mentioned above.

#### Pollinator exclusion experiments

Selective exclusion experiments were used to test whether *K. uvaria* is reliant on a pollinating vector for seed production and whether birds contribute significantly to seed production. To test for overall reliance on pollinators, exclusion bags were placed over ten individuals of *K. uvaria* - at the bud stage to ensure no pollinator visitations had taken place (Brown *et al*, 2009). The bags (30 cm x 15 cm) were made of a soft, porous material, which prevented both birds and insects from gaining access to flowers. Each bag had a drawstring that closed around the stem of the plant to ensure the bag was tightly closed (Hargreaves *et al*, 2004; Brown *et al*, 2009; Botes *et al*, 2009). Additionally, cages (mesh size: 19 mm x 21 mm) were placed over each of ten budding individuals (Hargreaves *et al*, 2004; Brown *et al*, 2009; Botes *et al*, 2009) at least 5m apart. This mesh size excludes birds but not most insects, including bees (Hargreaves *et al*, 2004; Brown *et al*, 2009; Botes *et al*, 2009). All individuals were marked with a standard nursery plant tag and given a number from one through to ten, for each treatment. The experiment ensued until each plant set fruits but was terminated before seeds were dispersed. As a positive control, for both the caged and bagged plants, we randomly selected ten individuals for

an open-pollination treatment (not bagged or caged) allowing visitation from all potential pollinators.

For each of the caged, bagged and open treatments, five mature fruits were removed from each individual, and stored in separate, labelled brown bags or envelopes to keep them dry.

For each of the three treatments (cage, bag and control) percentage fruit set was recorded for each individual by dividing the number of bracts present (a measure of the original number of flowers) by the number of fruits set by the plants (Brown *et al*, 2009).



**Figure 2:** Exclusion experiments depicting (a) a bagged plant and (b) a caged plant. (c) shows a section of the study site once bags and cages had been placed.

Seed set was determined, for each treatment (bagged, caged and open control), by counting the number of viable and non-viable seeds produced by each fruit. Seeds were classed as viable if they were plump and fleshy and classed as non-viable if they were flat and desiccated (see figure 7). The number of viable, non-viable and total number of seeds was recorded and compared for each treatment to determine pollination success for each. Flowers which did not set fruit were also included in the analyses for seed set.

The difference between viable and non-viable seeds can be seen in figure 3. The viable seeds were larger and fleshier compared to non-viable seeds.



**Figure 3:** Seeds of *K. uvaria*. The seed on the left is classified as non-viable and the seed on the right as viable.

### Statistical analyses

For pollinator observations, mean visitation rates and mean number of floral tubes probed per inflorescence for birds and insect were calculated and compared using a t-test (done in STATISTICA). The number of fruits and percentage fruit set were not normally distributed and therefore a non-parametric test was used to analyse the data. The Kruskal-Wallis test followed by multiple comparison tests were implemented in STATISTICA for each parameter to compare data between treatments. STATISTICA uses a Bonferroni method (see Siegel & Castellan, 1988 pp 213 – 215) for multiple comparison tests. The number of viable seeds and total number of seeds per plant were normally distributed and parametric tests were used to analyse the data. A one-way ANOVA followed by a Tukey HSD test were implemented in STATISTICA for each parameter to compare data between treatments.

## **Results**

### Pollinator observations

Throughout the study both specialist nectar feeding bird species and generalist opportunistic nectar feeding birds were observed visiting *K. uvaria* and probing flowers, along with bees (*Apis mellifera*) (Fig. 1 & 4). Frequent observations were made for the Malachite, Amethyst, Southern Double-collared, Greater Double-collared and Collared sunbirds (Table 1). The Greater Double-collared sunbird was also observed, but not as frequently as it is one of the rarer sunbird species for the study site. The observations for the opportunistic nectar feeding bird species, the Cape White-eye, and Cape Weaver, were much less frequent compared to the specialist bird species, with the exception of the Red-winged Starling.

There was evidence of nectar robbing on some of the flowers of *K. uvaria*'s inflorescences, which is an indication that some insects and birds possibly removed nectar without pollinating. During observations, the three generalist bird species were observed making contact with the reproductive parts of the flowers they visited.

**Table 1:** Observations of birds visiting *K. uvaria* inflorescences.

<b>Bird species</b>	<b>Number of birds observed</b>
<b><i>Specialist nectarivore species</i></b>	
Malachite sunbird ( <i>Nectarinia famosa</i> )	10
Amethyst sunbird ( <i>Chalcomitra amethystina</i> )	10
Southern Double-collared sunbird ( <i>Cinnyris chalybeus</i> )	8
Greater Double-collared sunbird ( <i>Cinnyris afer</i> )	6
Collared sunbird ( <i>Hedydipna collaris</i> )	5
<b><i>Opportunistic nectarivore species</i></b>	
Red-winged starling ( <i>Onychognathus morio</i> )	7
Cape White-eye ( <i>Zosterops capensis</i> )	4
Cape Weaver ( <i>Ploceus capensis</i> )	4



**Figure 4:** Visitation of insects to *K. uvaria*. A bee with pollen sacs (circled in red) on its legs approaching an inflorescence.

Observations indicated that birds visited more flowers per inflorescence than bees (Table 2) when visiting *K. uvaria*.

**Table 2:** Bird visitation rates versus bee visitation rates of *K. uvaria* flowers.

<b>Observation</b>	<b>Birds</b>	<b>Bees</b>	<b>t-test</b>
Mean ( $\pm$ SE) number of flowers visited per inflorescence	1.55 $\pm$ 1.56 (n=20)	1.39 $\pm$ 2.08 (n = 40)	t = -2.11; p < 0.05

### Flower and nectar measurements

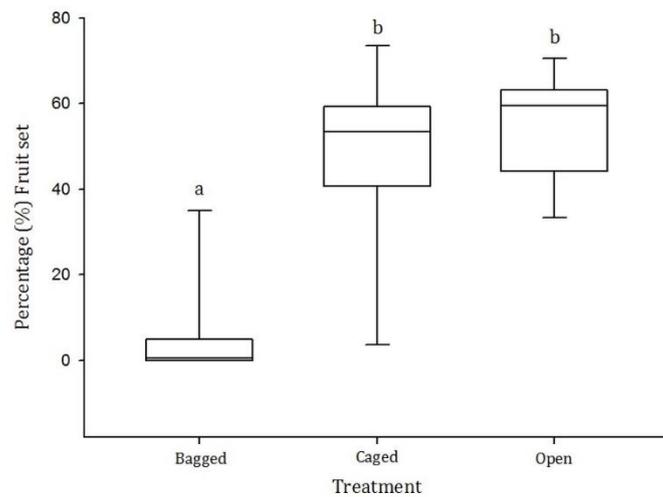
The mean volume of standing crop nectar was found to be lower than that of the 24hr nectar (Table 3), t-test: t = -7.00; p < 0.005. The mean percentage sugar concentration was found to be 12 percent.

**Table 3:** Floral and nectar mean measurements with standard errors.

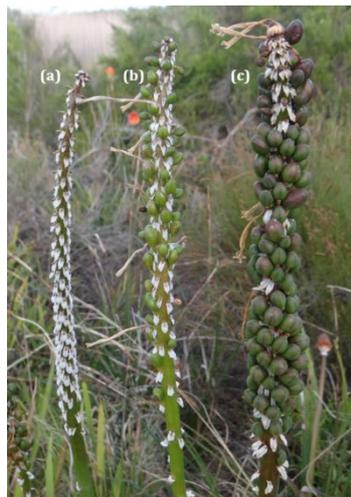
<b>Floral</b>		
<b>Measurement</b>	<b>Mean (<math>\pm</math>SE)</b>	<b>N</b>
Length (mm)	37.71 $\pm$ 0.23	197
Width (mm)	4.73 $\pm$ 0.04	197
<b>Nectar</b>		
Standing crop ( $\mu$ l)	4.22 $\pm$ 0.41	57
24hr ( $\mu$ l)	10.95 $\pm$ 1.06	57
Sugar concentration	12.15 $\pm$ 0.40	57

### Fruit set

There was a significant difference found between treatments overall (Kruskal-Wallis  $H_{2;30} = 16.8$ ; p < 0.001) (Fig. 5). The bagged treatment had a significantly lower percentage fruit set than the caged (p < 0.005) and open (p < 0.001) treatment (Fig. 6).



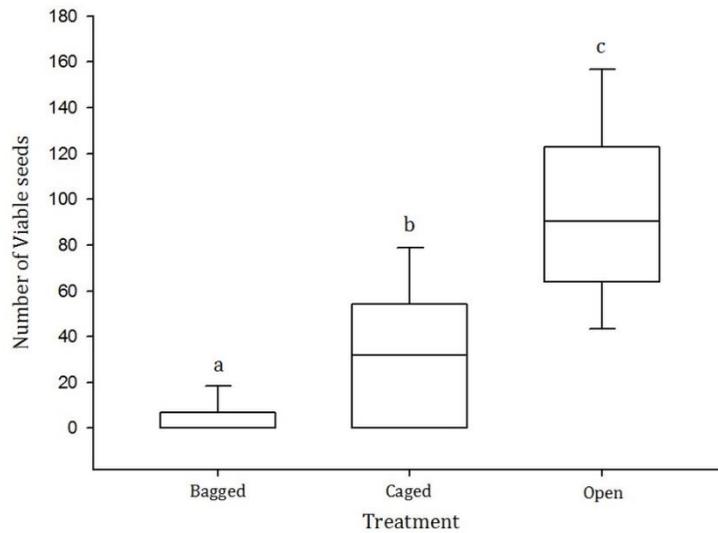
**Figure 5:** The effect of pollinator exclusion on percentage fruit set in *K. uvaria*. Different letters show a significant difference. Box plot show medians and quartiles.



**Figure 6:** The difference in fruit set between typical (a) bagged, (b) caged and (c) open inflorescences.

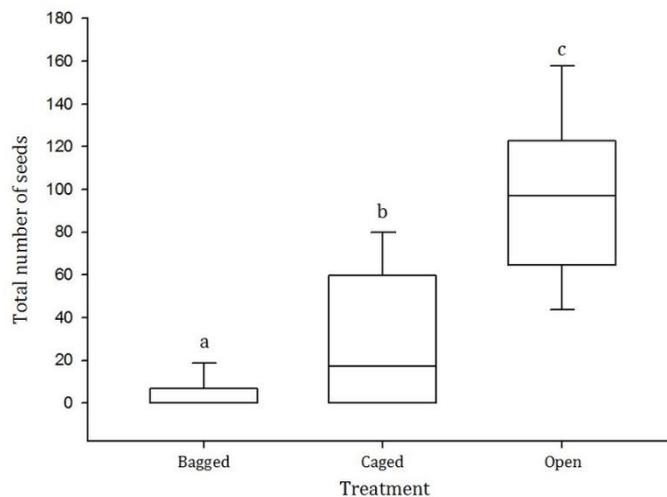
### Seed set

There was a significant difference between all treatments for the number of viable seeds (one-way ANOVA) (Fig. 7) per fruit. Seed set in bagged flowers was significantly lower than in caged flower ( $F = 27.6$ ;  $df = 2$ ;  $p = 0.014$ ) and open flower ( $F = 27.6$ ;  $df = 2$ ;  $p < 0.001$ ) treatments, and there was a significantly higher viable seed set in the open treatment compared to the caged treatment.



**Figure 7:** Difference between the bagged, caged and open treatments for the number of viable seeds produced in *K. uvaria*. Different letters show a significant difference. Box plots show medians and quartiles.

The bagged treatment contained significantly fewer seeds in total compared to the caged ( $F = 27.0$ ;  $df = 2$ ;  $p < 0.005$ ) and open treatments ( $F = 27.0$ ;  $df = 2$ ;  $p < 0.001$ ) and there was a significantly more seeds in total for the open treatment compared to the caged treatment (Tukey:  $p < 0.001$ ) (Fig. 8).



**Figure 8:** The difference between the bagged, caged and open treatments for the total number of seeds per fruit (viable and non-viable). Different letters show a significant difference. Box plots show medians and quartiles.

## Discussion

This study found that *K. uvaria* is likely to be a bird-pollinated species for this area. The flower structure (tubular) and colour (orange) of *K. uvaria* is consistent with the characteristics of the bird-pollination syndrome (van der Pijl, 1961; Hargreaves *et al*, 2004). Several sunbird species, most commonly Malachite, Amethyst and Southern double-collared sunbirds have been witnessed feeding on the flowers and pollen being deposited on the head, along with some Red-winged Starlings, Cape White-eyes and Cape Weavers acting as occasional nectar feeders (personal observations). The family Asphodelaceae contains several bird-pollinated species. *Kniphofia caulescens* is pollinated by short-billed, opportunistic nectar feeders, most notably the Drakensberg siskin (Brown *et al*, 2009). *Aloe marlothii* is pollinated by occasional nectar feeding birds (Symes & Nicolson, 2008; Symes *et al*, 2008) as is *Aloe vryheidensis* (Johnson *et al*, 2006). A study done by Brown *et al* (2010) looked at the pollination system for *Kniphofia laxiflora* and found this species to be pollinated by sunbirds, specifically Amethyst (*Chalcomitra amethystina*) and Malachite (*Nectarina famosa*). Another species of *Kniphofia*, *Kniphofia linearifolia*, is visited by both occasional nectar-feeding birds (Village weaver, *Ploceus cuculatus*), specialist nectar feeding birds (Malachite sunbird) and bees (Brown *et al*, 2011). The mountain pride butterfly (*Aeropetes tulbaghia*) has been documented as playing a role in the pollination of some populations of *K. laxiflora* (Johnson *et al*, 2009) and also obtains nectar in mountain populations of *K. uvaria* (Newman *et al*, 2012), however it did not seem to have a major role in this coastal population of *K. uvaria* (where the butterfly does not occur). One can not exclude the possibility that other butterfly species could play a role in pollination for this population and studies should be considered to investigate this possibility.

When comparing the nectar data (Table 2) with those for known bird-pollinated species (Johnson & Nicolson, 2008) we found it to be within the range of specialist bird-pollinated species. Plant species which are pollinated by specialist bird species, such as sunbirds, generally have a lower nectar volume (between 10 – 30  $\mu$ l) and higher concentration (between 15 – 25%) compared to those pollinated by generalist bird species (Johnson & Nicolson, 2008). Plant species which are pollinated by opportunistic nectar feeding bird species have a nectar volume of between 40 – 100  $\mu$ l and a concentration between 8 – 12% (Johnson & Nicolson, 2008). Nectar volume for *K. uvaria* was measured between 5 –

25 µl with a concentration of between 12 – 15 % with a few exceptions being lower or higher. Standing crop nectar volume was significantly lower than 24-hour nectar volume suggesting a high visitation rate throughout the day by multiple floral visitors. Based on the observations of bird species which visited *K. uvaria* and probed the flowers for nectar (Table 1), the presence of Red-winged starlings suggests a lack of sucrose within the nectar. Brown *et al* (2012) did a study on Red-winged starlings, *Onychognathus morio*, and their sugar preferences. It was found that the starlings are not capable of digesting sucrose, and preferred hexose sugar solutions rather than sucrose solutions (Bizaaré *et al*, 2012; Brown *et al*, 2012). Anecdotal evidence suggests the family Asphodelaceae is phylogenetically constrained with no sucrose present in any nectar studied so far.

The exclusion experiments were designed to determine the pollination effectiveness of insects and birds by excluding one visitor group and then allowing both to visit flowers (Hargreaves *et al*, 2004, Botes *et al*, 2009; Brown *et al*, 2009). Bagged flowers did not set any fruit (Fig 4), indicating that *K. uvaria* may not be autogamous (i.e. is not capable of autonomous self-fertilisation), as was found for some other species of *Kniphofia* (see Brown *et al*, 2009 and Brown *et al*, 2010). The fruit set for the open treatment was similar to the caged treatment, suggesting that insects and birds may play an equal role in pollination, however, seed set (see below) suggests otherwise. Fruit set should not solely be used as a proxy for pollinator effectiveness. Bagged and caged plants often set fruit, but these fruits do not always carry viable seeds (personal observation). It is, therefore, important to look at seed set, and the number of viable seeds compared to non-viable seeds per fruit for each treatment.

Seed set was higher for the open control treatments when compared to the caged treatment. Insects evidently did play a role in pollinating *K. uvaria*, but this was far less when compared to the role of birds. Flowers accessible by birds had a higher number of viable seeds and total number of seeds compared to those from which birds were excluded (Fig. 5). A study by Brown *et al* (2009) included similar exclusion experiments to determine pollinator effectiveness in *Kniphofia caulescens* and found similar results, which helped determine that *K. caulescens* was in fact bird pollinated. A study on *Protea roupelliae* (a bird-pollinated species) to test pollinator effectiveness also found that flowers where birds could visit had a higher seed set compared to flowers where birds were excluded (Hargreaves *et al*, 2004). A study by Duffy *et al* (2013) did emasculation

experiments on *Kniphofia linearifolia* and found that plants where pollinators were excluded did not produce any fruits. They also further confirmed bird pollination of *K. linearifolia* with emasculated plants still being visited by birds, compensating for lack of visitation by bees, and allowing for no difference in fruit set between emasculated and non-emasculated plants (Duffy *et al*, 2013).

Although this study provides evidence for birds playing an important role in the pollination of *K. uvaria*, it is experimentally difficult to exclude insects whilst allowing for bird visitation. Therefore, the exact contribution of birds to pollination cannot be measured. Fruit set is more a measure of pollinator quantity and gives an indication that visitation is taking place. The seed set per fruit allows the quality of the pollination that is occurring to be determined. Some other factors which could potentially alter the results of this study is that we did not test for self-compatibility properly with the use of controlled hand-pollination experiments. We also did not test for per-visit effectiveness (Johnson and Wester, 2017).

The fruit data which showed that bagged individuals did not set fruit, which in turn gives an indication that the *K. uvaria* may not be capable of selfing, could give insight into the risk this plant species may face should its pollinator become lost from the system. If *K. uvaria* is unable to self and finds itself in a system where its pollinator is no longer around, it could lead to *K. uvaria* being vulnerable and at risk to also being lost from the system.

## **Conclusion**

The results of this study suggest that *K. uvaria* is pollinated by birds. Based on observations of visitation and probing of floral tubes by Malachite, Southern Double-collared, Greater Double-collared, Collared and Amethyst sunbirds, we can further suggest that sunbirds (specialist nectar feeding bird species) are important for the pollination success of this plant species. Generalist bird species were observed less often, but when observed made contact with the reproductive part of the flower, which suggests that they may play a role in pollination. Further support for adaptation to birds comes from the floral and nectar traits of this species which fall within the bird-pollination guild.

Further studies need to be done for *K. uvaria* to help add to the understanding the pollination of this species. This includes analysing the nectar properties of *K. uvaria* nectar to see if there is an absence of sucrose, given that Red-winged starlings have been

observed feeding on its nectar. Hand pollination studies will help confirm that *K. uvaria*, and potentially other *Kniphofia* species, are not capable of autogamy. Germination trials would also be useful to determine if the seeds considered viable are in fact viable. The role that generalist bird species might play in pollination should also be more closely investigated as well as the possibility for butterfly pollination.

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## **Chapter 3**

# Evidence for bird-pollination in the Cape geophyte

## *Chasmanthe aethiopica* (Iridaceae)

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### **Abstract**

Much of the current evidence for bird pollination in the Iridaceae is derived mainly from observations of bird visitation, rather than from experimentation. We studied the pollination system of *Chasmanthe aethiopica* (Iridaceae) to determine whether it is pollinated predominantly by birds. We did exclusion experiments and compared fruit and seed set across different treatments. Standing crop and 24-hour nectar measurements were taken and compared to other bird pollinated syndromes. Exclusion of birds resulted in the production of fewer viable seeds, and smaller fruits. Nectar traits were found to be similar to those of other plants with specialist bird pollination systems. Based on the findings for this study we conclude that *C. aethiopica* is pollinated primarily by specialist nectarivorous birds.

Key words: *Chasmanthe aethiopica*, nectar, pollination, seed set

### **Introduction**

Characteristics of pollination syndromes include floral shape, floral colour, scent, rewards and floral structure (Hargreaves *et al*, 2004). Examples of pollination syndromes include reptile (Olsen & Valido, 2003), bird (van der Pijl, 1961; Hingston & McQuillan, 2000), mammal (van der Pijl, 1961; Hingston & McQuillan, 2000), and insect (van der Pijl, 1961; Hingston & McQuillan, 2000) pollination. One of the most studied of these pollination syndromes is the bird-pollination syndrome (Waser, 1978; Schemske, 1980; Frost & Frost, 1981; Johnson & Brown, 2004; Johnson *et al*, 2006; Brown *et al*, 2009; Geerts & Pauw, 2009; Geerts & Pauw, 2012). Flowers which use birds as their pollinators tend to have characteristics which include lively colours such as red and orange, pollen and nectar rewards, flowers with a tubular shape, diurnal anthesis

(exposure of anthers during the day) and flowers with no smell (van der Pijl, 1961; Hingston & McQuillan, 2000).

This relationship between plants and their pollinators is under threat because of the use of pesticides (Bond, 1994; Kearns & Inouye, 1997); fragmentation of natural habitat (Harris & Johnson, 2004); introduction of invasive species (Bond, 1994; Ghazoul, 2004) and the alteration of natural habitat (Bond, 1994; Kearns & Inouye, 1997). Bird pollinated plants are also susceptible to nectar robbing due to their tubular flower shape (Irwin & Maloof, 2002) where both birds and insects remove nectar without pollinating (Irwin & Brody, 1998). This is done by making slits (birds) or chewing holes (insects) in the flower tube to gain access to the nectar (Irwin & Brody, 1998). It is important to study these relationships as it helps not only to understand the part pollinators play in the diversification of plants (Johnson & Steiner, 2003) but also aids in conservation planning (Johnson & Steiner, 2003).

The genus *Chasmanthe* belongs to the Iris family (Iridaceae) and are commonly known as Cobra lilies (Manning, 2007). They are cormous perennials with leaves that form a basal fan, are sword-shaped, and display orange flowers (Manning, 2007). *Chasmanthe* species are endemic to the winter-rainfall areas of South Africa (Duncan, 2001) with their range from the south-western Cape, southern Cape and south-eastern part of the Eastern Cape (Duncan, 2001). There are three species, all of which are found in the fynbos, namely *Chasmanthe aethiopica*, *Chasmanthe bicolor* and *Chasmanthe floribunda* (Duncan, 2001; Manning, 2007). Of these three species, *C. aethiopica* has had little attention with only three studies found looking at its biology (Goldblatt *et al*, 2004; Bernhardt & Goldblatt, 2006; Goldblatt & Manning, 2006). Goldblatt *et al* (2004) looked at the biology, classification and cultivation of *Crocasmia* and *Chasmanthe*, with their focus being on cultivation. Goldblatt & Manning (2006) did an observational study over a number of irid species to look at the radiation of pollination systems within the family. The first study did not have a main focus on the pollination system of *Chasmanthe*, and although the second study did pollinator observations, where they found birds to frequently visit inflorescences, no exclusion experiments were done to test for the pollination system. A study by Geerts (2016) studied *Chasmanthe floribunda* and did some exclusion experiments (bagging only) while looking at pollination efficiency of short-billed, nectar thieving sunbirds. It would be useful to do more in depth studies into the pollination

system of the three *Chasmanthe* species. A study by van Kleunen *et al* (2008) compared the ability of self-fertilization between naturalized and non-naturalized irid species, and found *Chasmanthe floribunda* to be capable of autonomous self-fertilization. This suggests the potential for all *Chasmanthe* species to be self-compatible and auto fertile.

For this study, we wanted to determine the pollination biology of *C. aethiopica* by using exclusion experiments and collecting floral and nectar data to compare with known bird pollination systems (Johnson & Nicolson, 2008). We predicted that *C. aethiopica* was (a) bird pollinated, (b) had nectar measurements comparable to those of known bird-pollinated species and (c) potentially able to self-fertilize (bagged plants will produce fruit).

## **Material and methods**

### Study site

This study was conducted in the residential area of Nature's Valley (33° 58' 50" S; 23° 33' 33" E) which is surrounded by the Tsitsikamma section of the Garden Route National Park.

### Plant species

*Chasmanthe aethiopica* (also known as the cobra lily) is a member of the iris family (Iridaceae) and has a flowering period from April through to July. The flowers are orange in colour and tubular in shape (Fig. 1). *Chasmanthe aethiopica* grows to a height of approximately 40-65 cm. with sword-shaped leaves and orange flowers which grow in a spike (Manning, 2007). The species is patchily distributed within the study area, occurring in natural habitat patches, and along road-side edges.

### Pollinator observations

Observations were done to determine which type of pollinator frequents the species for its nectar. The pollinator observations were done for three consecutive days for a two-hour period in the morning (07:00 am – 9:00 am). Observations were done on a large flowering patch with observers stationed approximately 20m away from visiting birds and 1m from insect visitors. The number of flower tubes probed per inflorescence by birds and insects and the time spent on a single plant before moving to the next was recorded. Where possible, photos were taken of visitors.

### Floral and nectar measurements

Flowers removed from the plant for nectar samples were used for floral measurements. Due to the shape of the *C. aethiopica* flowers, three measurements were taken using digital callipers. Corolla length 1 was measured from the base of the ovary to the top of the lip (total length) and corolla length 2 was measured from the base of the ovary to the lower opening of the floral tube, which sits underneath the flower. The width of the flower was measured where the flower tubed splits into its petals. This was done for three floral tubes per individual (27 individuals, N=81) Floral measurements were taken to compare them to other known bird-pollinated plants species. All the floral measurements were taken using a digital calliper (Grip 150mm digital Vernier Calliper).

Standing crop nectar was extracted and measured using a 5 microliter ( $\mu$ l) capillary tube. This was done for three floral tubes per individual plant (total of 12 individuals, N=36). The volume was recorded and approximate sugar concentration of the nectar determined by means of a refractometer (ATAGO).

A 1 ml. insulin syringe was used to extract all nectar from three flowers on each of 12 individual plants (N=36). These tubes were marked, with string, and bagged (to prevent visitation) for 24 hours to determine nectar production over that period. Nectar secreted after 24 hours was measured as described above.

### Pollinator exclusion experiments

We examined pollinator efficiency by placing bags and cages over individual plants and comparing resultant fruit and seed set between treatments.

#### Bags and Cages

Plastic wire cages (mesh size: 19 mm x 21 mm) were placed over ten budding individuals to exclude bird pollinators. (Hargreaves *et al*, 2004; Brown *et al*, 2009; Botes *et al*, 2009). Additionally, bags were placed over ten individual plants, at the bud stage, to exclude all pollinators (Brown *et al*, 2009). The bags (30 cm x 15 cm) were of a soft, porous material and were tightly closed around the stem with a drawstring weaved through the bag opening (Hargreaves *et al*, 2004; Brown *et al*, 2009; Botes *et al*, 2009). Treated individuals were labelled, with bags and cages remaining on the plants until fruits were matured but before seeds were released. As a control treatment, we randomly selected ten individuals

which remained natural within the population to compare with the bagged and caged treatments.

### Fruit collection

For each of the treatments the entire stem with fruits was removed. Fruits were removed between 1.5 and 2 weeks after being set by the plant. Each fruit was stored in separate, labelled brown envelopes to keep them dry. The fruit set for each treatment (cage, bag and control) was recorded by counting the number of bracts present (a measure of the original number of flowers) and dividing by the number of fruit set by the plants.

### Seed set

We allowed for the fruits to dry before removing the seeds to determine seed set for each of the treatments. Once dry the fruits were cut open and the number of viable and non-viable seeds were counted for each treatment (bagged, caged and open). Viable seeds were plump and fleshy and non-viable seeds were flat and not fleshy. The number of viable, non-viable and total number of seeds was recorded and compared for each treatment to determine pollination success.

### Statistical analyses

Mean visitation rates and mean number of floral tubes probed per inflorescence for birds and insect were calculated along with the standard error, for both. Mean floral length and width, nectar volume for standing crop and 24hr nectar measurements and mean percentage nectar sugar concentration, were along with the standard deviation for all measurements. The number of fruits and fruit set percentage data were not normally distributed and non-parametric tests were used to analyse these data. A Kruskal-Wallis test followed by multiple comparison tests were implemented in STATISTICA for each parameter to compare data between treatments. STATISTICA uses a Bonferroni method (see Siegel & Castellan, 1988 pp 213 – 215) for multiple comparison tests. The number of viable seeds data were not normal; however, the total number of seeds data were normally distributed. As not all data were normal, non-parametric test were used to analyse these data. A Kruskal-Wallis test followed by multiple comparison tests were implemented in STATISTICA for each parameter.

## Results

### Pollinator observations

Frequent observations were made throughout the study of sunbird visitation on *C. aethiopica* (Fig. 2) (Table 1). Greater Double Collared, Southern Double Collared and Amethyst sunbirds were all frequent visitors and probing flowers. No insects were observed visiting flowers during the pollinator observations. Exact numbers are not shown in the table as many of the populations were lost. Numbers in the table represent an estimated number of personal observations over a two-year period on a population within view of our place of work.

Table 1: Observations of birds visiting *C. aethiopica* inflorescences. c. refers to estimates

<b>Bird species</b>	<b>Number of birds observed</b>
<b><i>Specialist species</i></b>	
Southern Double-Collared sunbird ( <i>Cinnyris chalybeus</i> )	c. 10
Greater Double Collared sunbird ( <i>Cinnyris afer</i> )	c. 20
Amethyst sunbird ( <i>Chalcomitra amethystina</i> )	c. 10



Figure 1: A Southern Double-collared sunbird drinking nectar from a floral tube of *C. aethiopica*. Note position of anthers on bird's head (a), and subsequent possible pollen deposition with anthers touching head (b).

### Floral and nectar measurements

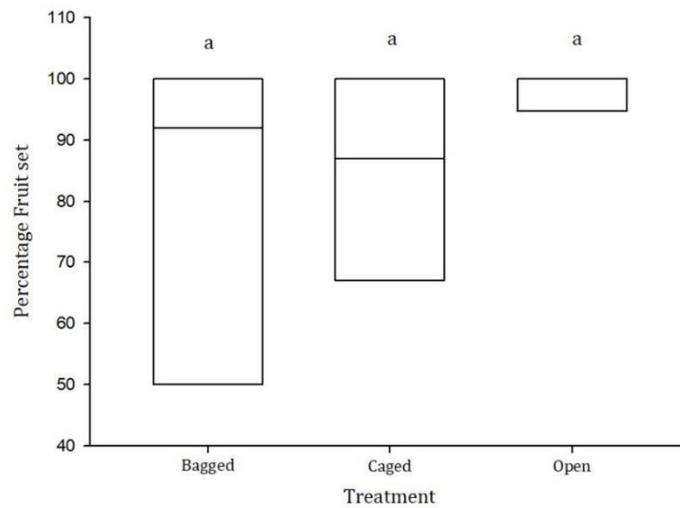
The mean volume of standing crop nectar was found to be lower than that of the 24hr nectar (Table 2), t-test:  $t = -7.00$ ;  $p < 0.05$ . The mean percentage sugar concentration was found to be 16 percent.

Table 2: Floral and nectar mean measurements with standard errors.

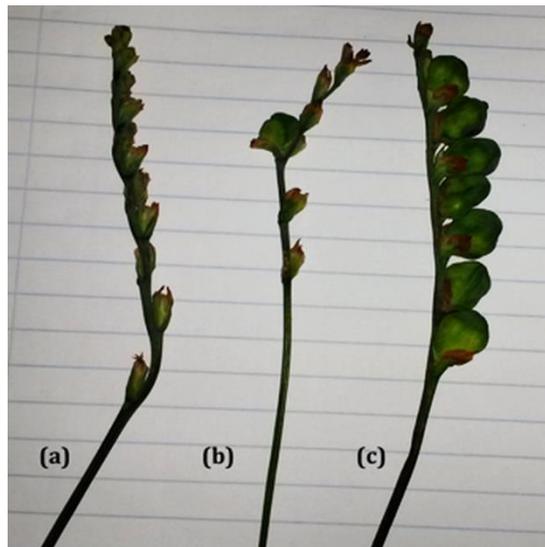
<b>Floral</b>		
<b>Measurements</b>	<b>Mean (<math>\pm</math>SE)</b>	<b>N</b>
Length 1 (mm)	47.71 $\pm$ 0.40	81
Length 2 (mm)	26.05 $\pm$ 0.46	81
Width (mm)	5.87 $\pm$ 0.13	81
<b>Nectar</b>		
Standing crop ( $\mu$ l)	4.60 $\pm$ 0.51	36
24hr ( $\mu$ l)	9.67 $\pm$ 1.66	14
Sugar concentration (%)	16.97 $\pm$ 1.51	14

### Fruit set

There was no significant difference found between treatments for the percentage median of fruit set (Kruskal-Wallis  $H_{2;16} = 3.934$ ;  $p > 0.05$ ) (fig. 3).



**Figure 2:** The difference between the bagged, caged and open treatments for the percentage fruit set. Different letters show a significant difference. Box plots show medians and quartiles.

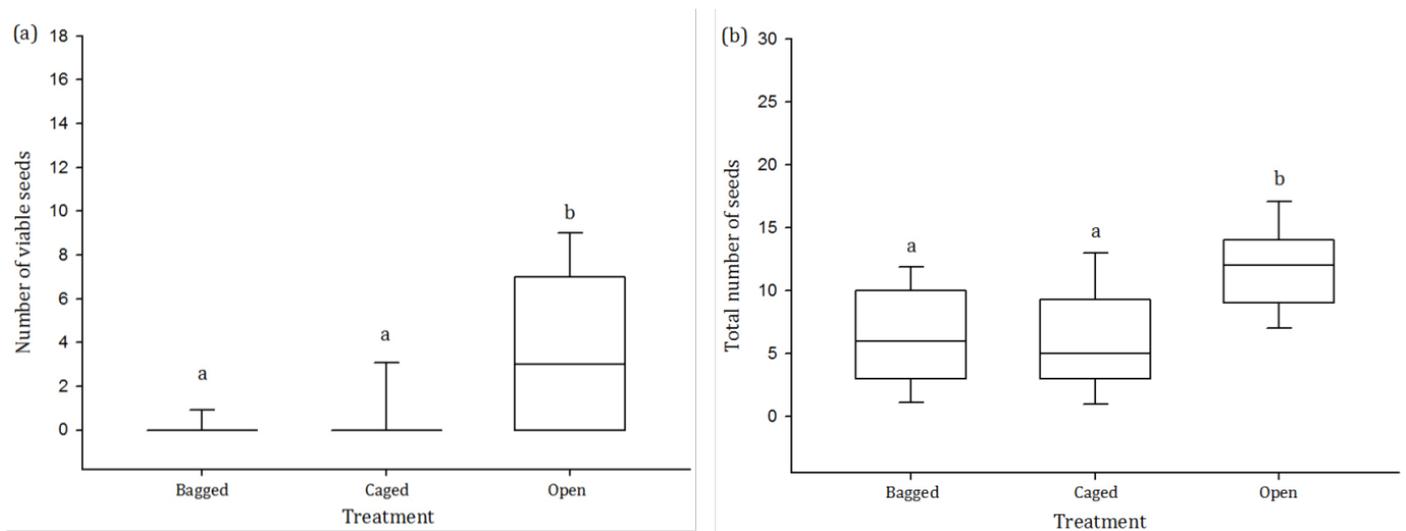


**Figure 3:** The difference in number of fruits and fruits size for the (a) bagged, (b) caged and (c) open inflorescences.

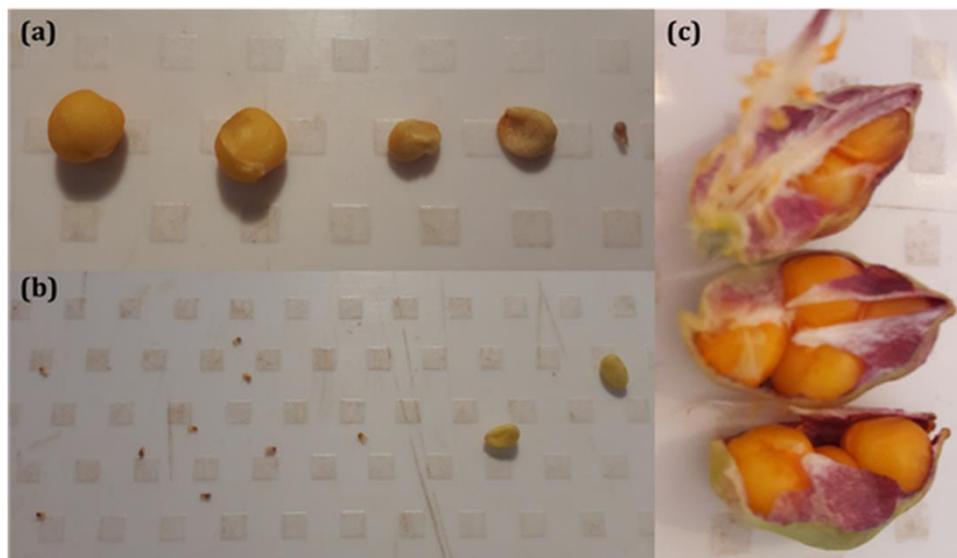
**Seed set**

The median number of viable seeds were significantly different between treatments (Kruskal-Wallis  $H_{2; 176} = 38.6$ ;  $p < 0.001$ ) (Fig. 5a). The open control had a significantly higher median number of viable seeds compared to the caged ( $p < 0.001$ ) and the bagged treatments ( $p < 0.001$ ).

Total median number of seeds per fruit showed a significant difference between treatments (Kruskal-Wallis  $H_{2,176} = 14.8$ ;  $p < 0.05$ ). The open treatment had a significantly higher total median number of seeds compared to the bagged ( $p < 0.05$ ) and caged ( $p < 0.05$ ) treatments (Fig. 5b). See figure 6 for difference in seeds for the treatments.



**Figure 4:** The difference between the bagged, caged and open treatments for (a) the number of viable seeds per fruit and (b) the total number of seeds per fruit. Different letters show a significant difference. Box plots show medians and quartiles.



**Figure 5:** Difference in seed size for *C. aethiopica*. (a) seed size for an open treatment (first two seeds), caged treatment (third & fourth seeds) and bagged treatment (last seed). (b) seeds removed from the fruit of a caged plant and (c) fruits with seeds of a control plant.

## Discussion

This study supports the hypothesis that *C. aethiopica* is pollinated by specialist avian nectarivores. Nectar volume generally ranged between 11 – 15  $\mu\text{l}$  and sucrose concentration between 14 – 19 % which falls into the range for specialist bird-pollinated species (10 – 30  $\mu\text{l}$  and 15 – 25 %; Johnson & Nicolson, 2008). Goldblatt *et al* (2004), suggested that all three species of *Chasmanthe* are bird-pollinated and found that they had sucrose concentrations of 10 – 17 % which is within the same range as our study. Standing crop nectar volume was much lower compared to 24-hour nectar volumes (Table 3), suggesting a high visitation rate to flowers, with only sunbirds being observed visiting flowers during the study.

Fruit set appeared to be similar across all treatments (Fig. 3). However, total number of viable seeds and overall number of seeds was significantly different (Fig. 5) between treatments. This is an indication that percentage fruit set, on its own, is not a good measure for pollination effectiveness. Geerts (2006) and van Kleunen *et al* (2008) have reported *C. aethiopica* to be autogamous. Germination trials of seeds collected from fruit of bagged plants are needed.

The number of viable seeds was similar for the bagged and caged treatments with a much higher number found for the open treatment. This suggests that firstly, insects play little to no role in pollination and consequently fertilization, and secondly, birds are the main (possibly exclusive) pollinator for this species. The total number of seeds showed that the open treatment had more “seeds” (it is possible that not all were seeds but undeveloped ovules) compared to the bagged, with caged having a higher number of seeds as well. A study by Geerts and Pauw (2009) looked at the Malachite sunbird pollination syndrome. *C. aethiopica* was one of their study plant species and was found evidence of visitation by sunbirds and possible pollination (Geerts & Pauw, 2009).

The Southern double-collared (*Cinnyris chalybeus*) and Dusky (*Cinnyris fuscus*) sunbirds are important bird-pollinators for Iridaceae found in coastal habitats along western southern Africa (Goldblatt & Manning, 2006). During the study, we observed Southern Double-collared sunbirds, Greater Double-collared sunbirds (*Cinnyris afer*) and Amethyst sunbirds (*Chalcomitra amethystina*), feeding on the nectar of *C. aethiopica*. These

observations of birds frequenting *C. aethiopica* suggests evidence that this plant species makes use of a bird-pollination system.

## **Conclusion**

We concluded that due to the significant differences between seed size and fruit size between the bagged, caged and open treatments, with the open treatment having a significantly larger fruit and seed size, that *C. aethiopica* is bird pollinated. The nectar and floral morphology data gathered also supports our claim that *C. aethiopica* is indeed bird-pollinated. Although the bagged treatments yielded fruit, there was little to no viable seed within the fruit therefore we suggest that *C. aethiopica* is not capable of autonomous self-fertilization.

Future studies for this species should include the sugar composition of the nectar for this genus of iris' and controlled hand-pollination to confirm whether *C. aethiopica* is not capable of self-fertilization (i.e. is genetically self-incompatible). Nectar robbing should be more closely investigated as short-billed sunbirds would be expected to rob.

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## **Chapter 4**

# Tests for Allee effects in patches of *Kniphofia uvaria* and *Chasmanthe aethiopica*

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### **Abstract**

The continued fragmentation of the landscape due to urbanization and development causes habitat disturbance and loss and may greatly impact plants and their dependents. We studied patchily distributed populations of the geophytic plant species *Kniphofia uvaria* and *Chasmanthe aethiopica* to determine if there were Allee effects present. For each patch the number of plants, percentage fruit set and seed set were documented, and these data were compared between patches and distances between patches. Univariate regression results showed a significant positive linear relationship between patch size and fruit set in *K. uvaria*, but no significant relationship for seed set, and a significant negative linear relationship between inter-patch distance and fruit set. *Chasmanthe aethiopica* showed a significant negative linear relationship between inter-patch distance and average percentage fruit set, with no relationship found for seed set. Patch size in *C. aethiopica* had no significant relationships for both fruit and seed set. Multiple regression models showed a significant effect of patch size and patch distance on fruit set in *K. uvaria*, but no significant effect of these variables on seed set. In the case of *C. aethiopica*, inter-patch distance showed a significant negative effect on fruit set. It was concluded that apart from the effect of patch size on fruit set in *K. uvaria*, this population could potentially exhibit an Allee effect, whereas marked Allee effects were not evident in the population of *C. aethiopica*.

Key words: *Allee effect, Chasmanthe aethiopica, distance, Kniphofia uvaria, patches*

### **Introduction**

Currently, the main threats to rare plant species and vegetation types include habitat fragmentation (Lundberg & Moberg, 2003; Johnson, 2004; Pauw & Louw, 2012); land alteration (Gess & Gess, 1993; Kearns & Inouye, 1997); introduction of invasive species (Ghazoul, 2004) and the change in climate (Hannah *et al*, 2002; Dawson *et al*, 2011; Wise

*et al*, 2012). These alterations of the landscape can cause plant populations to become more fragmented and patchy in distribution. Patchiness, in turn, can lead to a population experiencing a phenomenon known as the Allee effect. Allee (1938) first coined the idea of an Allee effect which he observed in small plant populations. The Allee effect refers to a decrease in population growth because of low population numbers (Allee *et al*, 1949; Cappuccino, 2004).

There are two recognized types of Allee effects, component and demographic. The component Allee effect is the relationship between population density and individual fecundity (Stephens *et al*, 1999; Deredec & Courchamp, 2007; Gregory *et al*, 2010). The demographic Allee effect refers to the relationship between the number of individuals within a population and the growth rate of that population (Stephens *et al*, 1999; Deredec & Courchamp, 2007; Gregory *et al*, 2010). The potential causes that have been suggested for a Allee effect to occur include extinction (Dennis, 2002; Boukal & Berec, 2002); spread of parasites through the population (Deredec & Courchamp, 2006); establishment of a population (Drake & Lodge, 2006; Liebhold & Tobin, 2006); a variation within metapopulations (Zhou *et al*, 2004; Martcheva & Bolker, 2007) and the interaction occurring between predators and their prey (Gascoigne & Lipcius, 2004; Morozov *et al*, 2004). The main causes of an Allee effect within a plant population are mate limitation (Agren, 1996; Ashman *et al*, 2004; Cheptou & Avendano, 2006; Busch & Schoen, 2008) and reduced attractiveness of small populations to pollinators (Agren, 1996; Agren *et al*, 2008; Dauber *et al* 2010).

Larger population sizes have a benefit over smaller populations due to a decreased chance of inbreeding; better success for reproduction and more pollination opportunities (Stephens *et al*, 1999). Smaller populations are at greater risk of extinction; lower reproductive success; a lower number of suitable mates and fewer pollinators (Stephens *et al*, 1999; Bossuyt, 2007; Levin *et al*, 2009). In general, habitat fragmentation is expected to lead to smaller population sizes. A study done by Aizen and Feinsinger (1994) compared smaller forest fragments to larger, continuous forest patches. The level of pollination and fruit and seed set data were collected for all the patches and compared (Aizen & Feinsinger, 1994). They found that smaller forest fragments had much lower pollination events and fruit and seed set when compared to the larger, continuous patches (Aizen & Feinsinger, 1994). However, in some cases, patch size has been shown

to have little to no effect on pollination rates or reproductive success (e.g. Wilson *et al* 2009), suggesting that Allee effects do not always occur when expected. In this chapter, we ask whether Allee effects apply to patches (clusters) of plants within a broader genetic population. By patch size we mean the number of plants in a spatially distinct cluster. Pollinators would be expected to assess such clusters in terms of profitability of foraging and thus small patches would be predicted to have lower fecundity due to decreased pollinator visitation and also lower plant mate availability.

In plant populations patch sizes could have an impact on pollination success. Small patches are more prone to Allee effects which lead to low seed set mainly due to pollen limitation (Kunin, 1993; Ghazoul, 2005; Leimu *et al*, 2006). A large patch is more at risk for increased competition and a dilution effect on pollinators (Zimmerman, 1980; Johnson *et al*, 2012; Ward *et al*, 2013; Marini *et al*, 2014). Based on studies looking at smaller and larger patches, one could infer that patches of a medium size may provide more optimum conditions for a maximum pollination rate by having a balance between (1) attractiveness to pollinators and (2) a reduced swamping or dilution effect of too many flowers. This study investigated the potential Allee effect in two plant species, *Kniphofia uvaria* and *Chasmanthe aethiopica*.

Our predictions for this study were that that plant fecundity (fruit and seed production) would increase in relation to patch size (number of plants in a cluster), and be negatively affected by the distance to other patches.

## **Materials and methods**

### Study site

The site for *K. uvaria* was located within the Tsitsikamma section of the Garden Route National Park in the Western Cape of South Africa. The R102 (33° 57' 57.33" S; 23° 32' 36.05" E), which links Nature's Valley to the N2, has a population of *K. uvaria* growing along its verges. This population is patchily distributed along this road and flowering plants are not found away from the roadside. This may result from increased water availability due to runoff from the road.

The site for *C. aethiopica* was based in the municipal suburb of Nature's Valley (33° 58' 50" S; 23° 33' 33" E). Throughout the residential area *C. aethiopica* can be found growing, with a patchy distribution.

### Plant species

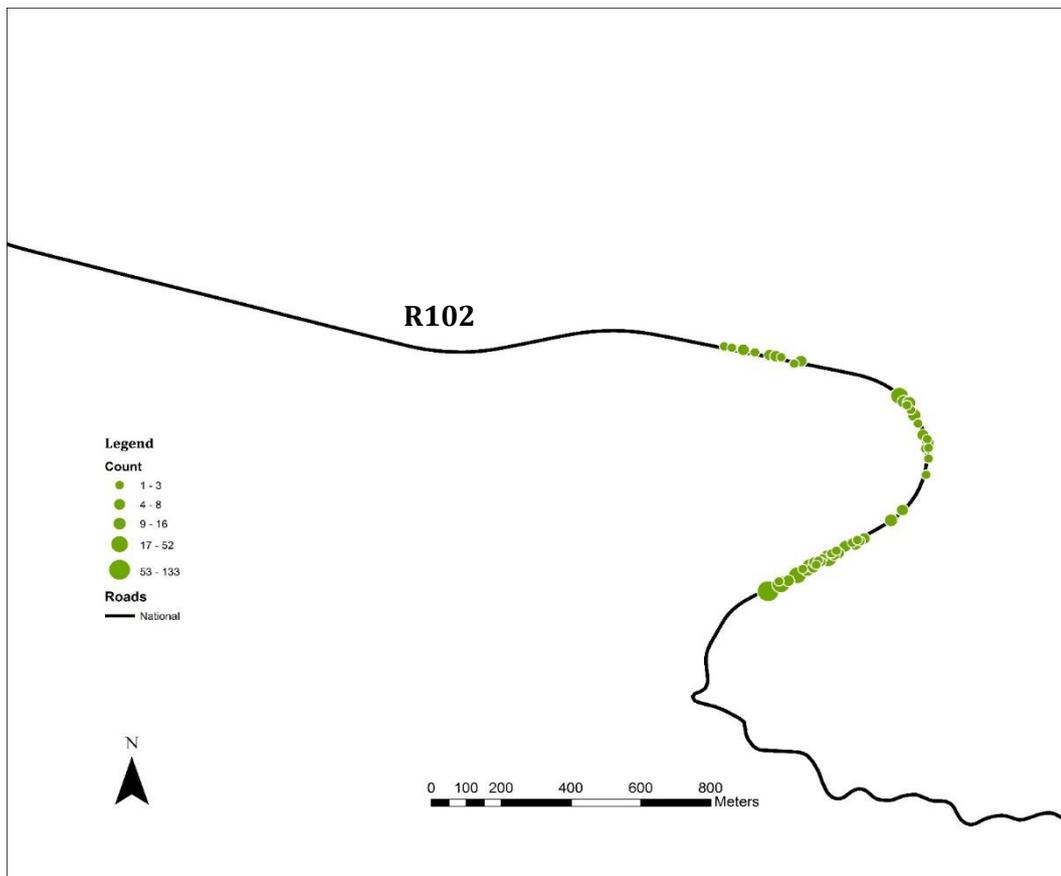
*Kniphofia* is a genus belonging to the Asphodelaceae family which is comprised of species found within Africa, southern Arabia and New Zealand (Manning, 2007). This genus is characterized by soft (sometimes fibrous) leaves which are channelled and narrow (Codd, 1968; Manning, 2007). The flowers form an inflorescence which can be either white, yellow and red shades of colour and tubular in shape (Codd, 1968; Manning, 2007).

*Chasmanthe* is a genus belonging to the iris family (Iridaceae) and has a range from the southwestern Cape through to the Eastern Cape (Duncan, 2001; Manning, 2007). This species is perennial which grows between 40cm to 65cm in height, has soft leaves and orange, tubular flowers (Manning, 2007).

### Patches

#### *Kniphofia uvaria*

Clumps of plants which were growing five metres or more apart were classed as individual patches. We assumed 5m would need a direct flight by a bird to another patch rather than a small movement between two neighbouring plants. Once patches were identified their exact location was then recorded using a GARMIN etrex 10. The start and end of each patch was marked, and the number of plants counted and recorded to determine the size of the patch. This was done for 2016 and 50 patches were identified with nine patches lost due to mowing of the verges.



**Figure 1:** Population map for the year 2016 for *K. uvaria*. Shows the distribution of the patches (50 total) along the R102 towards Natures Valley. Patch sizes are marked with different sized circles.

### *Chasmanthe aethiopica*

The GPS coordinates for all patches of *C. aethiopica* were recorded, throughout the municipal suburb, using a GARMIN etrex 10. A patch was identified as such only if there were at least five meters between plant groups. The start and end of each patch was then marked and the number of individuals counted for each patch. This was done for 2016 with 27 patches identified.

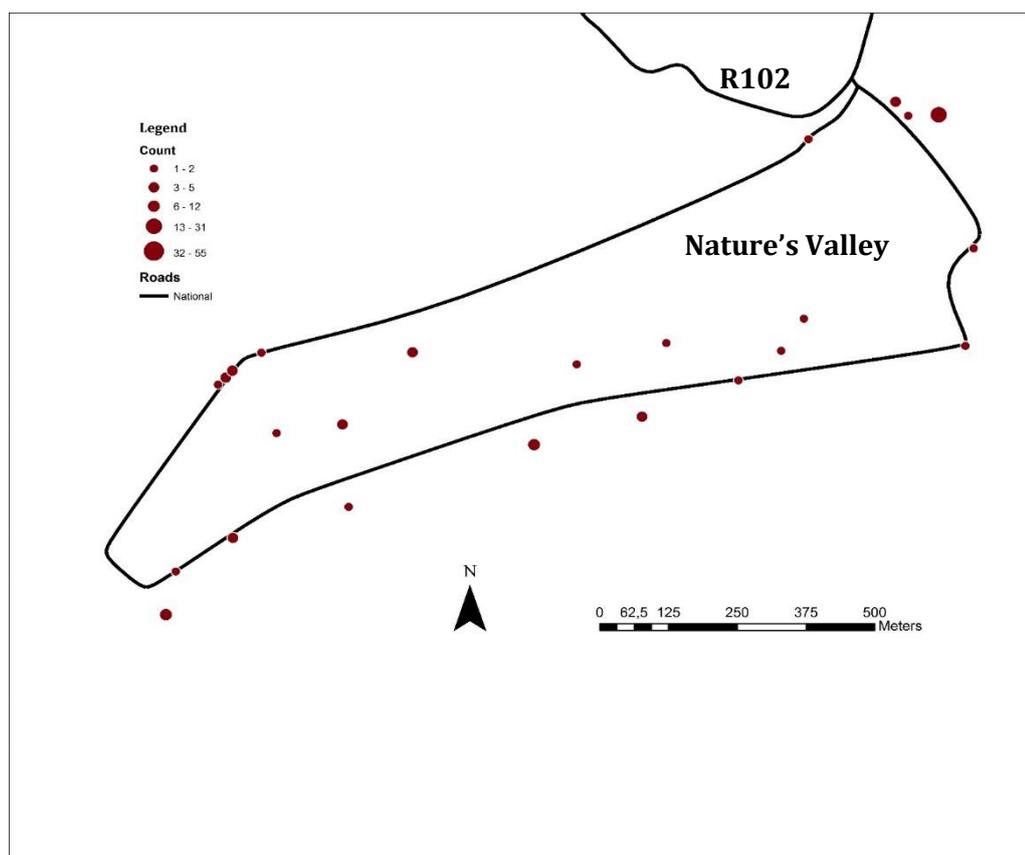


Figure 2: Population map for the year 2016 for *C. aethiopica*. Shows the distribution of the patches (27 total) throughout the residential area of Natures Valley. Patch sizes are marked with different sized circles.

### Maps

GPS data collected for 2016 for *C. aethiopica* and *K. uvaria* was used to generate population maps for each year using ArcGIS 10.2.2. The year 2015 was not included as the population had been mowed down by the Western Cape Roads Department before we could GPS the patches. The nearest neighbour distances for patches were also determined using ArcGIS 10.2.2. Using the "Closest Feature Distance" function from the ET Geowizards 11.3 extension for ArcGIS 10.2 (available online <http://www.ian-ko.com/>) the identity and distance to the closest patch was calculated for each patch per year. We felt distance to nearest neighbour was important as it would determine the amount of energy a bird would need to expend moving from patch to patch, and birds would therefore pollinate patches closer together rather than further apart.

### Fruit and seed set

After flowering had finished, we selected ten plants at random for each patch (for patches with less than ten individuals, all individuals were sampled). For each of these individual plants we counted the number of bracts (represents number of flowers) and number of fruit to determine the percentage fruit set. The number of fruits and percentage fruit set was then averaged for each patch. We then collected five fruits from each of the ten individuals. Each fruit was placed into its own brown paper bag which was labelled with the date, patch name and plant it came from. Once the fruits were dry we counted the number of viable and non-viable seeds in each fruit. Flowers which did not set fruit were not included in the analyses. Viable seeds were those which were plump compared to the non-viable seeds which were smaller and not plump. Once the seeds had been counted we averaged the number of viable seeds and total number of seeds to determine seed set per fruit for each patch.

### Statistical analyses

STATISTICA 13 (Dell Software, 2015) was used for all analyses. Variables used in regression models were log-transformed to improve normality of residuals.

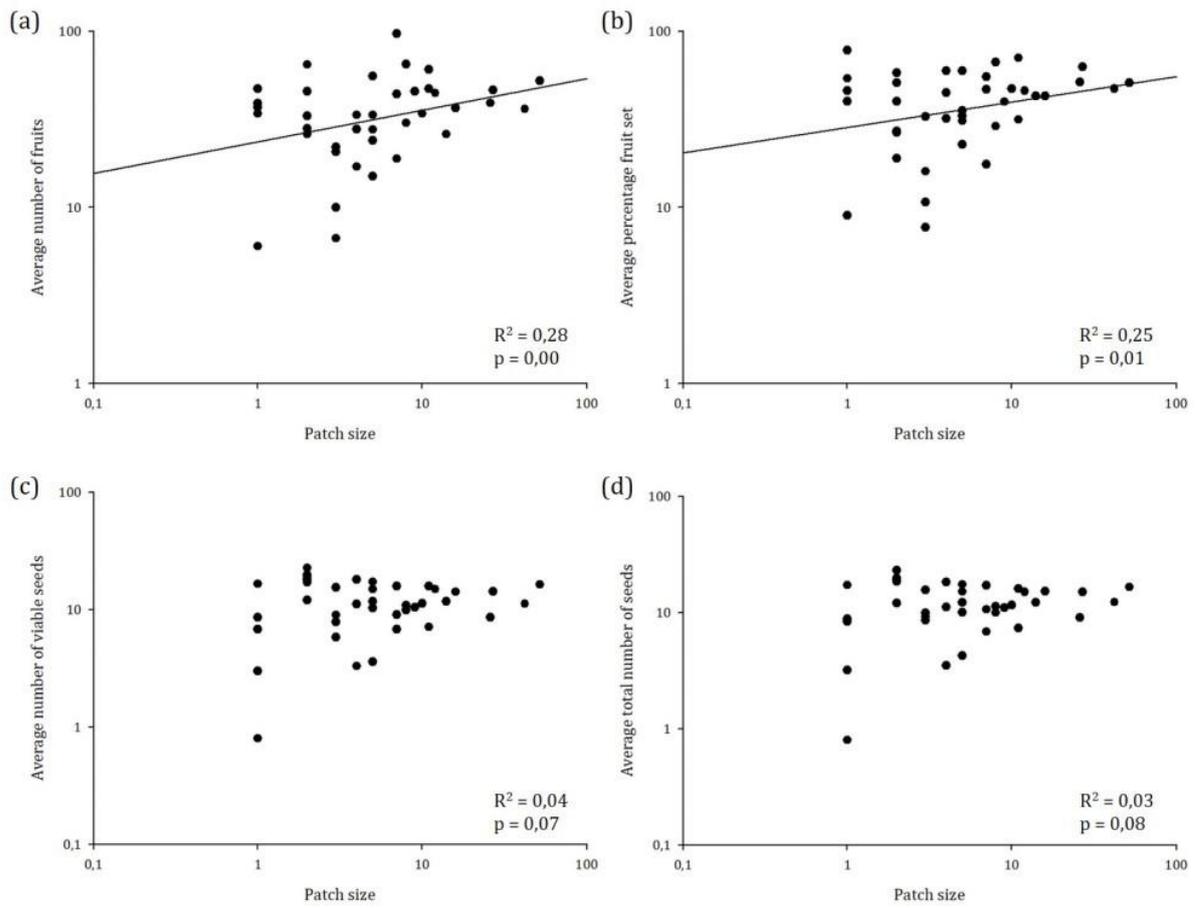
Univariate regression models were used for the graphs to illustrate relationships between the predictors patch size and distance to nearest neighbour, and response measures of fruit and seed set. This was done for both *K. uvaria* and *C. aethiopica*.

Multiple regression models were used for each species to compare relationships between the predictors, patch size and distance to nearest neighbour, and response measures of number of viable seeds, total number of seeds, number of fruits and percentage fruit set.

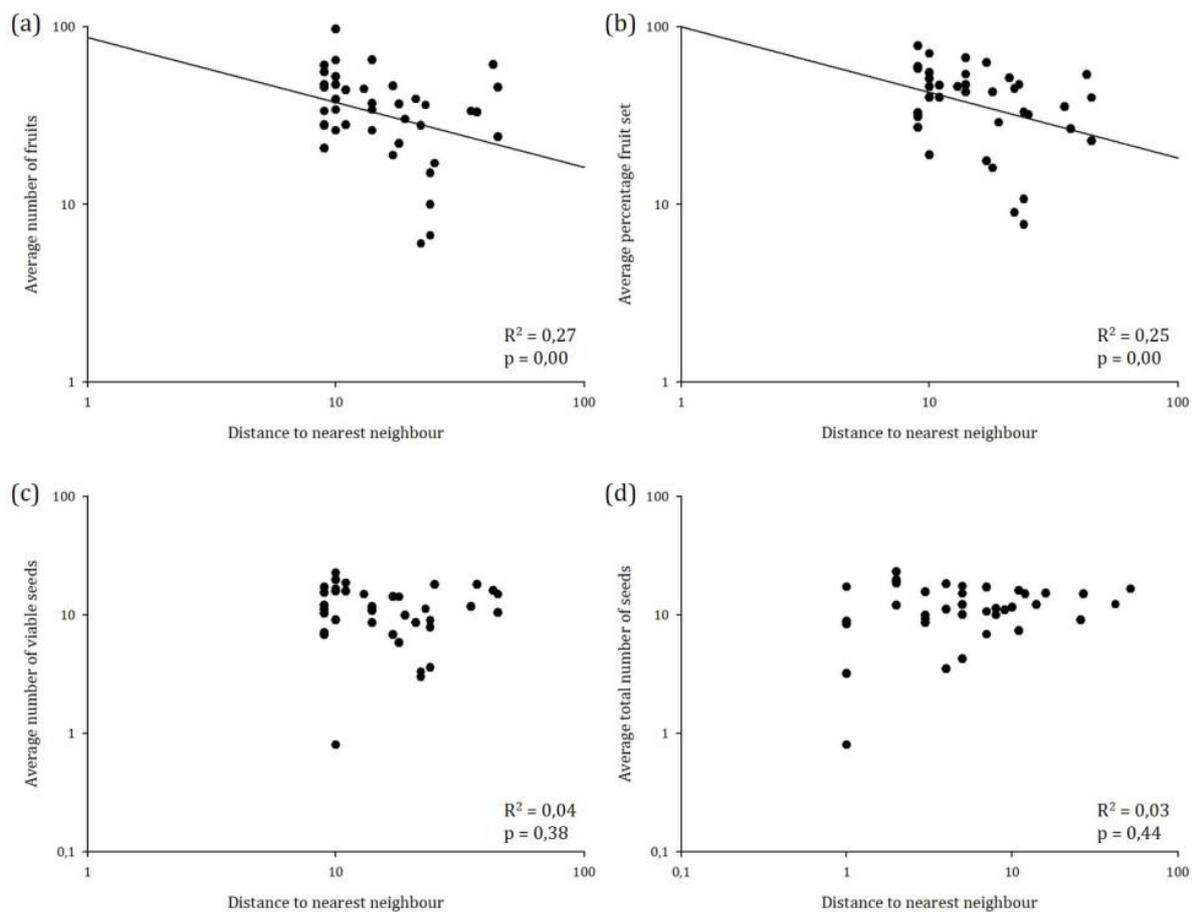
## **Results**

### *Kniphofia uvaria*

Based on univariate regression analyses, the average total number of fruits and percentage fruit set had a significant positive linear relationship with patch size (Fig. 3) and a significant negative linear relationship with distance between patches (Fig. 4). The number of viable seeds and total number of seed per plant showed no significant relationship between patches (Fig. 3) and the distance between patches (Fig. 4).



**Figure 3:** The relationship between patch size and (a) average number of fruits per plant, (b) average percentage fruit set, (c) average number of viable seeds per fruit and (d) average total number of seeds (viable and non-viable) per fruit for *K. uvaria*.



**Figure 4:** The relationship between distance to nearest neighbour and (a) average number of fruits, (b) average percentage fruit set, (c) average number of viable seeds per fruit and (d) average total number of seeds (viable and non-viable) per fruit for *K. uvaria*. Multiple regression model results were significant for the relationships between predictors patch size and distance to nearest neighbour, and the measure of fruits set (average number of fruits and percentage fruit set) (Table 1). The models were not significant for the relationships between the predictors and the measures of seed set per fruit (average number of viable seeds and average total number of seeds) (Table 1).

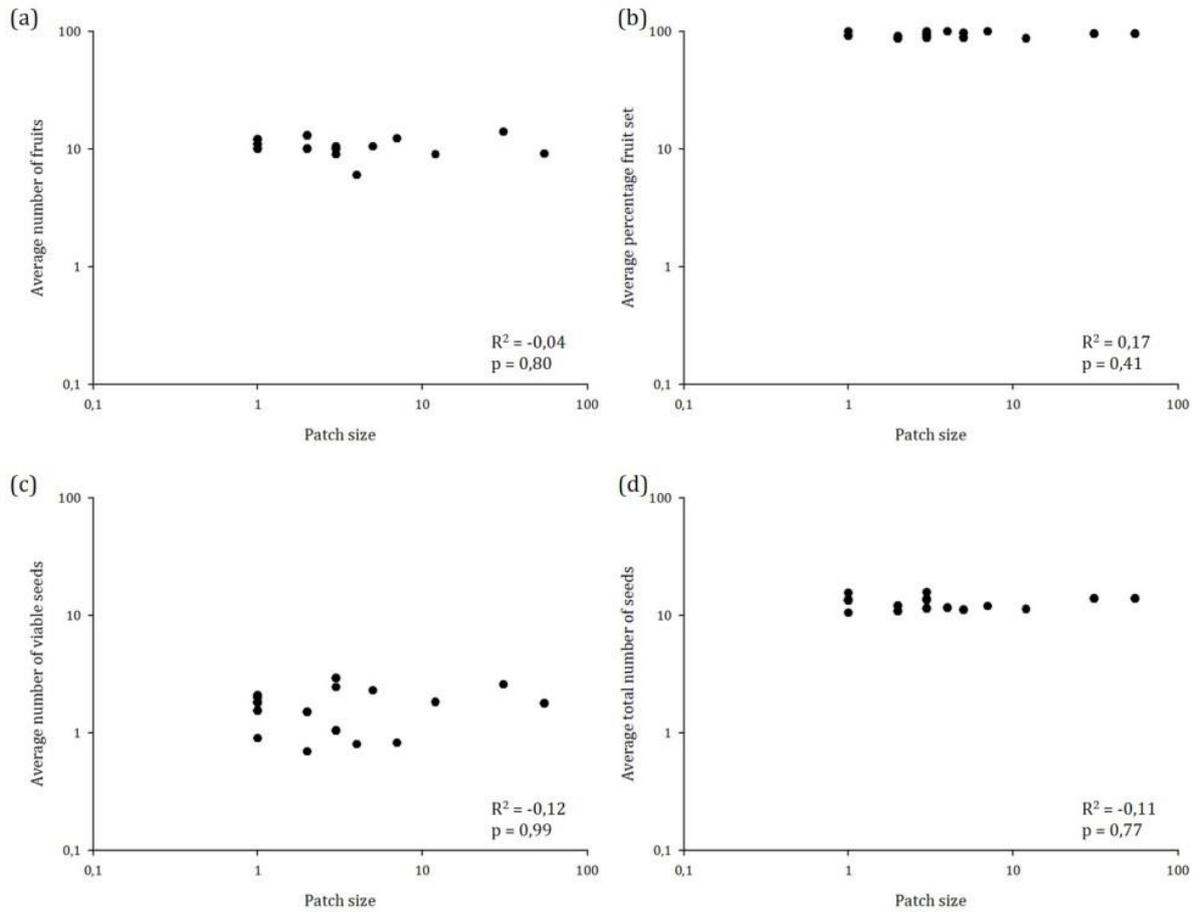
**Table 1:** Multiple regression models of the relationship between two predictor variables (patch size and distance to nearest neighbour) and measures of seed and fruit set for *K. uvaria*.

Response variables	Standardized partial regression coefficient		R <sup>2</sup>	Model	
	Patch size	Distance to nearest neighbour		F	P
Average number of fruits	0.479***	-0.452***	0.316	8.531	0.001
Average percentage fruit set	0.428***	-0.460***	0.288	7.478	0.002
Average number of viable seeds	0.306	-0.145	0.090	1.840	0.173
Average total number of seeds	0.299	-0.128	0.085	1.714	0.194

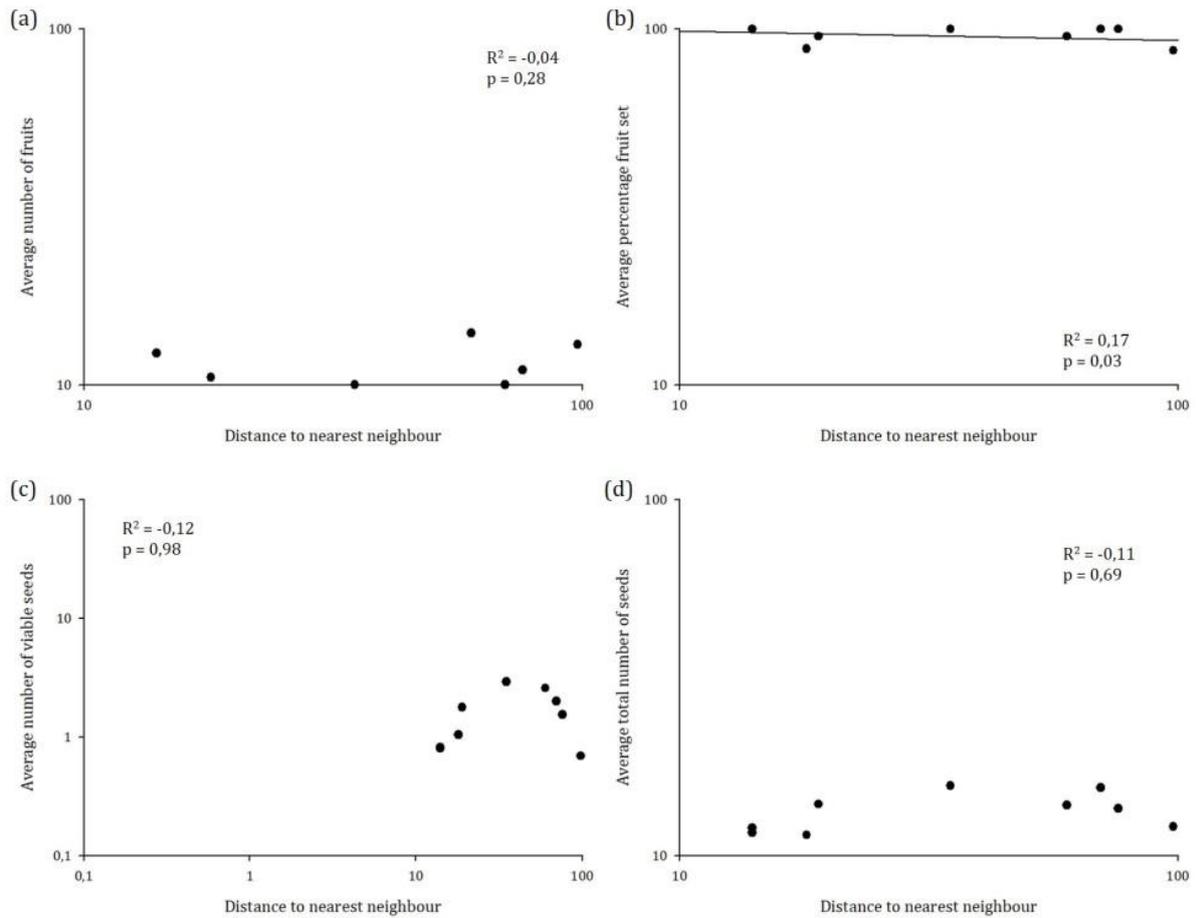
\*\*\* p < 0,01

#### *Chasmanthe aethiopica*

In univariate regression models, the average total number of fruits showed no significant relationship to patch size (Fig. 5a) or for distance between patches (Fig. 6a). Average percentage fruit set showed no significant relationship with patch size (Fig. 5b) but did show a significant negative linear relationship for distances between patches (Fig. 6b). The number of viable seeds and total number of seed per plant showed no significant relationship with patch size (Fig. 5c & 5d) and the distance between patches (Fig. 6c & 6d).



**Figure 5:** The relationship between patch size and (a) average number of fruits, (b) average percentage fruit set, (c) average number of viable seeds per fruit and (d) average total number of seeds (viable and non-viable) per fruit for *C. aethiopica*.



**Figure 6:** The relationship between distance to nearest neighbour and (a) average number of fruits, (b) average percentage fruit set, (c) average number of viable seeds per fruit and (d) average total number of seeds (viable and non-viable) per fruit for *C. aethiopica*.

Multiple regression models showed a significant effect of the predictor distance to nearest neighbour and the measure of average percentage fruit set, but not for the predictor of patch size for this measure (Table 2). The model showed no significant effect of the predictors on the measures of average number of viable seeds, average total number of seeds and average number of fruits (Table 2).

**Table 2:** Multiple regression models of the relationship between two predictor variables (patch size and distance to nearest neighbour) and measures of seed and fruit set for *C. aethiopica*.

Response variables	Standardized partial regression coefficient		R <sup>2</sup>	Model	
	Patch size	Distance to nearest neighbour		F	P
Average number of fruits	0.067	0.296	0.075	0.651	0.535
Average percentage fruit set	-0.202	-0.566***	0.264	2.873	0.086
Average number of viable seeds	-0.005	0.007	0.000	0.000	0.999
Average total number of seeds	-0.082	-0.113	0.012	0.094	0.910

\*\*\* p < 0,01

## Discussion

In testing for the presence of an Allee effect in different sized patches of plants, studies have found that smaller patch sizes tend to be at greater risk of lack of pollinator visits and production of viable seed compared to larger patches. In this study that trend was found for fruit set in *K. uvaria*, but not for *C. aethiopica*, with no significant relationships found between patches of different sizes and distances between patches for seed set per fruit for either species. Percentage fruit set per plant yielded a different result for both plant species with significant positive and negative linear relationships found. The differences found between *K. uvaria* and *C. aethiopica* could be a result of the linear distribution of the *K. uvaria* population compared to the patch distribution of *C. aethiopica*. It could be that with populations of *C. aethiopica* being so far spread from each other, birds were more likely to visit any plants they came across, leading to better fruit set compared to populations of *K. uvaria*.

*Kniphofia uvaria* showed a positive linear relationship between patch size and the average number of fruits (Fig. 3a) and average percentage fruit set (Fig. 3b) per plant. This suggests that as the size of a patch increases so does the number of fruits produced per plant. While patch size showed a positive linear relationship towards fruit set in *K. uvaria*, distance to nearest neighbour had a negative linear relationship (Fig. 4a & b). The

relationship between distance to nearest neighbour and the average percentage fruit set per plant in *C. aethiopica* showed another negative linear relationship (Fig. 6b). When looking at and comparing fruit set and seed set, fruit set reflects whether any visits from pollinators occurred and seed set reflects the amount of pollen that has been deposited. Considering fruit set over seed set would give a better understanding of whether smaller patches of flowering individuals are attracting fewer or equal numbers of pollinators compared to larger patches. Seed set would give an indication of the amount of successful pollen deposition and not necessarily of number of pollination visits or attempts.

These results for seed set per fruit could be an indication that the population of birds which pollinate *K. uvaria* and *C. aethiopica* is large enough to sustain patches of different sizes. With a large enough population of birds in the area, this could also explain why distances between patches had no effect on seed set. As we did not include plants which did not set fruit, we, by default, biased the results for *K. uvaria* and masked the Allee effect which showed in the fruit set but not the seed set. An example of a study where the pollination system was successful within a patchily distributed population of plants is a study by Coomb *et al* (2009). *Gamphocarpus physocarpus* is pollinated by a generalist wasp system which was enough to sustain both small and large patches with successful pollination (Coomb *et al*, 2009).

Another possibility could be that smaller patch sizes are as capable as larger patches in attracting sufficient numbers of pollinators for successful pollination to take place. A study by Wilson *et al* (2009) looked at the threatened *Aloe pruinosa*. They found no significant relationships between patch sizes and seed production and concluded that small patches were as successful as larger ones (Wilson *et al*, 2009). A study by Duffy *et al* (2013) did a study on *Kniphofia linearifolia* and found that population size, and not density or distance, was associated with an increase in bird abundance and seed set. They suggested that effective pollinators and their responses to plant aggregation could be a factor on Allee effects on plant fecundity (Duffy *et al*, 2013). The significant relationships found between patch size and distance for fruit set could have the explanation that as larger patches have more plants one would expect these patches to have a larger number of fruits compared to smaller patches. Essentially, it seems that even low visitation rates in small patches, while leading to reduced fruit set in *K. uvaria*, still lead to high fecundity for those individual flowers visited.

## Conclusion

In conclusion, there appears to be an Allee effect when considering patch size and distance to nearest neighbour for *K. uvaria*, however the effect is diluted to non-significant when looking at viable seeds counts. As we did not take into account plants which did not set fruit, we masked the potential Allee effect present for this species. Based on this we concluded that the population of *K. uvaria* may exhibit a potential Allee effect and the population of *C. aethiopica* did not exhibit an Allee effect. The relationship between the predictors and fruit set gives an indication that regardless of size or distance that patches may be sufficient at attracting pollinators. For mobile pollinators like birds, which have highly specialised pollination syndrome fits with the flowers they pollinate, Allee effects may not be prevalent in smaller more isolated populations.

Further studies need to be done on this population determine if this is an Allee effect at work. Data for fruit and seed set was collected for only one year of flowering, it might be useful to gather these data over multiple years. It may be useful to colour pollen and trap birds to take pollen samples to determine which patches are being frequented by birds. Pollinator observation for all patches would also be a good measure of how successful patches are at attracting the necessary pollinators.

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## **Chapter 5**

# Flowering phenology and seasonal incidence of nectarivorous birds in fynbos of the southern Cape.

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### **Abstract**

Understanding the plant phenology of an area and the pollination systems in place is important for conserving communities at the landscape level. We studied a section of the Tsitsikamma Section of the Garden Route National Park, covering two hiking trails, documenting the fynbos species and bird presence for the area. A total of 135 plant species were found and identified, with 14 species (c. 10%) potentially being primarily bird-pollinated. Data gathered through bi-weekly mist netting at both sites showed presence of sunbirds and sugarbirds match the times at which the plants they visit flowered. The ringing data showed that sugarbirds were not year-round members of the community assemblage. Sunbirds showed a year-round presence within the community.

*Key words: bird ringing, plant phenology, Tsitsikamma*

### **Introduction**

Phenology refers to the study of the timing of biological events in either animals or plants and can include flowering times, moulting, reproduction or growth (Lieth, 1974). In plant phenology, a term which is often used is phenophase, which refers to a certain stage of the plants growth which is limited to a certain time frame (Monasterio & Sarmiento, 1967). Plant communities are rife with competition for resources (water, nutrients, pollinators and seed dispersers to name a few) required for growth, seed dispersal and pollination (Pierce, 1984). Thus, resource partitioning lessens competition in communities allowing for co-existence between species and leading to higher diversity within the ecosystem (Pierce, 1984). A way in which plants can resource partition is by staggering their phenophases by either having different flowering times or presenting rewards at different times (Pierce, 1984). Other plant stages which could be staggered include leaf expansion, germination, seed set, fruiting and bud-bursts (Fenner, 1998).

Thomson *et al* (2000) looked at bee- and bird-pollinated plant species which flower together.

The Fynbos covers an area of approximately 90 000 km<sup>2</sup> (Goldblatt & Manning, 2002) and has over 9 000 species of which over 8 000 are flowering plants (Goldblatt & Manning, 2000; Goldblatt & Manning, 2002) showing how diverse this biome is. Other characteristics of fynbos include oligotrophic soils (Kruger, 1978); dependence on fire (Kruger, 1978) and distinct types of soil (i.e. clay, sandstone, limestone and quartzite, Goldblatt & Manning, 2002; van der Niet & Johnson, 2009). Kruger (1978) wrote of an east to west gradient that can be found across the fynbos biome with non-seasonal to winter rainfall patterns. Johnson (1992) also showed a winter rainfall pattern (south-western Cape) to non-seasonal rainfall (southern Cape) pattern along the fynbos biome. Pierce (1984) compared the growth of restios and found that there was trend for west to east, with earlier growth towards the east. The south-eastern Cape had an autumn, winter and spring growth phase (Pierce, 1984), southern Cape an early winter until spring growth phase (Bond, 1980) and the south-western Cape a spring and early summer growth phase (Kruger, 1981). After fire, ephemeral assemblages appear first, these include: Iridaceae, Orchidaceae (orchids) and Amaryllidaceae (amaryllis) (Geerts & Pauw, 2009). The Proteaceae and Ericaceae then follow with the late successional phase (Geerts & Pauw, 2009).

It is important to study the seasonal phenology of plants to help determine (1) the type of vegetation and its classification (Steenkamp *et al*, 2008), (2) the effect climate change is having on growth phases of plants (Reed *et al*, 2003), (3) monitoring desertification (Heumann *et al*, 2007), and (4) keeping track of changes in landscape and use of land (Steenkamp *et al*, 2008). Phenology has been used in several studies to determine community structure of pollination systems, and the effect of climate change on these interactions (Levin & Anderson, 1970; Stiles, 1977; Copland & Whelan, 1989; Eriksson & Bremer, 1992; Bolmgren *et al*, 2003; Memmott *et al*, 2007). Bolmgren *et al* (2003) studied the flowering patterns and species richness of plants pollinated abiotically and biotically. They found that niche partitioning (different flowering periods) was more important for biotically pollinated flowers (Bolmgren *et al*, 2003). Memmott *et al* (2007) looked at the potential effect of climate change on phenological shifts between plants and their pollinators. Their models found that these interactions would be disrupted with

flower resources not being available for pollinators at the right time (Memmott *et al*, 2007).

Birds play an important role in the pollination of fynbos groups such as proteas (Rebelo *et al*, 1984; Collins & Rebelo, 1987; Johnson *et al*, 2014) and ericas (Rebelo *et al*, 1984; Rebelo & Siegfried, 1985; Rebelo *et al*, 1985; Geerts & Pauw, 2009). Other plant families abundant in the fynbos which use birds as pollinators include Iridaceae (Manning & Goldblatt, 2005; Goldblatt & Manning 2006) and Asphodelaceae (Johnson *et al*, 2006; Symes *et al*, 2008). Plants which rely on specialised bird-pollinators have the following adaptations: floral parts are normally red in colour, contain nectar rewards and are tubular in shape (van der Pijl, 1961; Brown & Kodric-Brown, 1979; Faegri & van der Pijl, 1979; Johnson, 1996; Hingston & McQuillan, 2000; Johnson & Nicolson, 2008; Geerts & Pauw, 2009). Plant phenology can play an important role in structuring communities of nectar-producing plants and nectar-feeding birds communities and several studies done in Costa Rica (Stiles, 1975; Feinsinger, 1978; Feinsinger *et al*, 1986; Murray *et al*, 1987), Trinidad and Tobago (Feinsinger & Colwell, 1978; Linhart & Feinsinger, 1980 Linhart & Feinsinger, 1980) and Colorado (Waser, 1978) have shown this.

Thus, the purpose of this study was to (1) identify plant species and determine their flowering times and (2) draw a comparison of the presence of nectar-feeding birds with the flowering times of their dependent plants. We predicted that the (1) phenology of the area would be similar to that found elsewhere along the southern Cape; (2) there would be staggered flowering times within different families and (3) when birds are present so will their flower counterpart(s) be flowering and when they are no longer flowering, birds will be absent or less frequently seen in the area.

## **Materials and methods**

### Study site

The study was done within the De Vasselot section of the Tsitsikamma section of the Garden Route National Park. There are two hiking trails namely Kalander Kloof and Salt River. These areas link with the R102 (33° 57' 57.33" S; 23° 32' 36.05" E) which links Nature's Valley (33° 58' 50" S; 23° 33' 33" E) to the N2. Both hiking trails are areas dominated by fynbos vegetation and Afromontane forests.

### Plant surveying

Twice a month each hiking trail was sampled, and all flowering plants photographed and identified. Citizen Science platforms, such as iSpot and OrchidMap, were used to assist and confirm plant identifications. Flowering instances were recorded for each species every month, from May 2014 to September 2016. The phenology data collected were presence/absence based. Even if only one individual of a species was flowering it was marked off as such; the entire population's flowering percentage was not taken into account. The section of the Kalandar Kloof hiking trail used stretched over a distance of 4.8 km and the Salt River hiking trail 6.4 km. Plants were considered to be bird-pollinated based on their morphology and personal observations of birds visiting and making contact with the reproductive parts.

### Bird ringing

A long-term bird-ringing research program running on the two hiking trails was used for the phenology study. The ringing areas cover about 250m along the Eskom access road on the Kalandar Kloof hiking trail and another 250 m, from the picnic site towards the salt river mouth, on the Salt River hiking trail. Every two weeks (alternating between the two sites) twelve-meter by two and a half-meter mist nets (16 mm mesh size) are placed along the trail (twenty-one nets total i.e. 252 meters).

Each bird caught is processed with a ring and set of measurements prescribed by SAFRING (The South African Bird Ringing Unit) which is based at the University of Cape Town. The ring, with a unique number-letter combination, is placed onto the leg of each bird. Measurements included bill length, wing and tail length; moult stage; presence or absence of a brood patch and net weight. The birds are all safely released after being processed. Ringing data collected, from May 2014 to September 2016, was used to check if sunbird and sugarbird presence coincides with their flowering plant species. Based on their being nectar-feeding species, the Cape sugarbird, Orange-breasted sunbird and Southern Double-collared sunbird were the three focal species for this study.

### Statistical analysis

STATISTICA 13 (Dell software, 2015) was used to analyse data.

A Pearson's Chi-square test was used to compare the number of bird-pollinated and non-bird pollinated species present.

## Results

### Plant surveys

A total of 92 species (appendix 1) were identified for the Kalander Kloof hiking trail site, with Asteraceae, Fabaceae and Ericaceae the families making up a large percentage of the species composition (Table 1). Of the 92 species identified, 11 of those were plant species presumed to be primarily pollinated by birds (Table 2). These included three Ericaceae species, one Asphodelaceae species, four Proteaceae species and three Iridaceae species.

Table 1: The three families making up the most species found for Kalander Kloof.

#	Family	Number of genera	Number of species
1	Asteraceae	16	20
2	Ericaceae	1	11
3	Fabaceae	6	9

Table 2: Bird-pollinated plant species along the Kalander Kloof hiking trail.

Species	Family	Probable pollinator
<i>Erica densifolia</i>	Ericaceae	Sunbird
<i>Erica discolor</i>	Ericaceae	Sunbird
<i>Erica sessiliflora</i>	Ericaceae	Sunbird
<i>Kniphofia uvaria</i>	Asphodelaceae	Sunbird
<i>Leucospermum cuneiforme</i>	Proteaceae	Sugarbird
<i>Protea cynaroides</i>	Proteaceae	Sugarbird
<i>Protea neriifolia</i>	Proteaceae	Sugarbird
<i>Protea mundii</i>	Proteaceae	Sugarbird
<i>Tritoniopsis caffra</i>	Iridaceae	Sunbird
<i>Watsonia fourcadei</i>	Iridaceae	Sunbird
<i>Watsonia knysnana</i>	Iridaceae	Sunbird

The Salt River hiking trail site yielded a total of 89 species (appendix 2), with the Asteraceae, Ericaceae, Fabaceae, Iridaceae, Orchidaceae and Crassulaceae families making up a large percentage of the species composition (Table 3). There were eight plant species of the 89 which were identified as bird-pollinated. These included one

Rubiaceae species, one Crassulaceae species, one Amaryllidaceae species, two Ericaceae species, one Asphodelaceae species and two Proteaceae species (Table 4).

**Table 3:** The six families making up the most species found for Salt River.

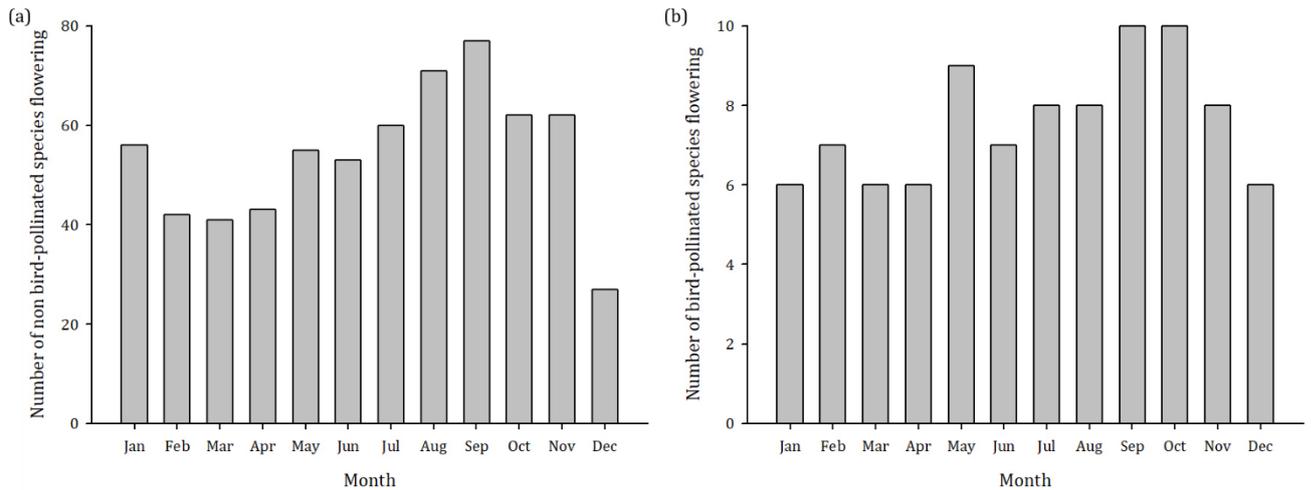
#	Family	Number of genera	Number of species
1	Asteraceae	11	13
2	Ericaceae	1	7
3	Fabaceae	6	7
4	Iridaceae	4	5
5	Orchidaceae	5	5
6	Crassulaceae	2	5

**Table 4:** Bird-pollinated plant species along the Salt River hiking trail.

Species	Family	Probable pollinator
<i>Burchellia bubalina</i>	Rubiaceae	Sunbird
<i>Cotyledon orbiculata subsp orbiculata</i>	Crassulaceae	Sunbird
<i>Cyrtanthus elatus</i>	Amaryllidaceae	Sunbird & Sugarbird
<i>Erica discolor</i>	Ericaceae	Sunbird
<i>Erica glandulosa sbsp fourcadei</i>	Ericaceae	Sunbird
<i>Kniphofia uvaria</i>	Asphodelaceae	Sunbird
<i>Leucospermum cuneiforme</i>	Proteaceae	Sunbird
<i>Protea neriifolia</i>	Proteaceae	Sugarbird

When comparing the species lists for the two study sites, each site shared 90 of the 136 species identified. The Kalandar Kloof site has two flower species which were not found at the Salt River site.

The peak flowering time for non bird-pollinated plant species (Fig. 1a) falls between July and November, with a drop in the number of flowering species during December. Flowering time for bird-pollinated species (Fig. 1b) is fairly constant throughout the year with a slight increase in the number of flowering species in September and October. The Pearson’s Chi-square test found no significant relationship between the number of bird-pollinated and non bird-pollinated species (p= 0.344).

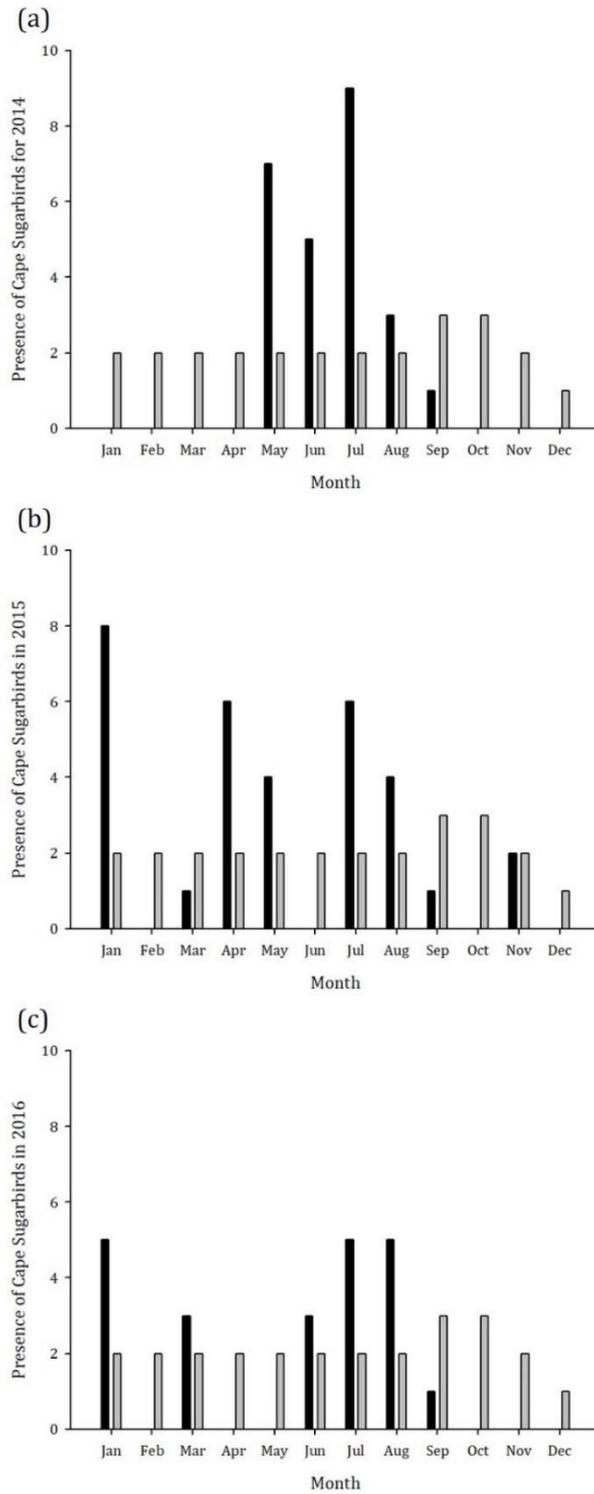


**Figure 1:** Flowering times for (a) non bird-pollinated and (b) bird-pollinated plant species.

### Bird ringing

The bird ringing database showed that sunbird and sugarbird species were present at the same time the flowers they visit were flowering. Data was extracted from the database starting May 2014 until September 2016.

Cape sugarbirds (*Promerops cafer*) were absent during February, October and December and in low numbers for March, September and November (Fig. 2). Four bird-pollinated protea species (predominantly sugarbird pollinated) occurred in the study area namely, *Leucospermum cuneiforme* (pin-cushion), *Protea cynaroides* (King protea), *Protea mundii* (White-forest sugarbush) and *Protea neriifolia* (Bearded sugarbush). Throughout the year proteas were in flower with *P. mundii* and *P. neriifolia* flowering for a longer period compared to *L. cuneiforme* and *P. cynaroides*. Figure 2 shows that Cape sugarbirds were present during the time proteas were in flower.



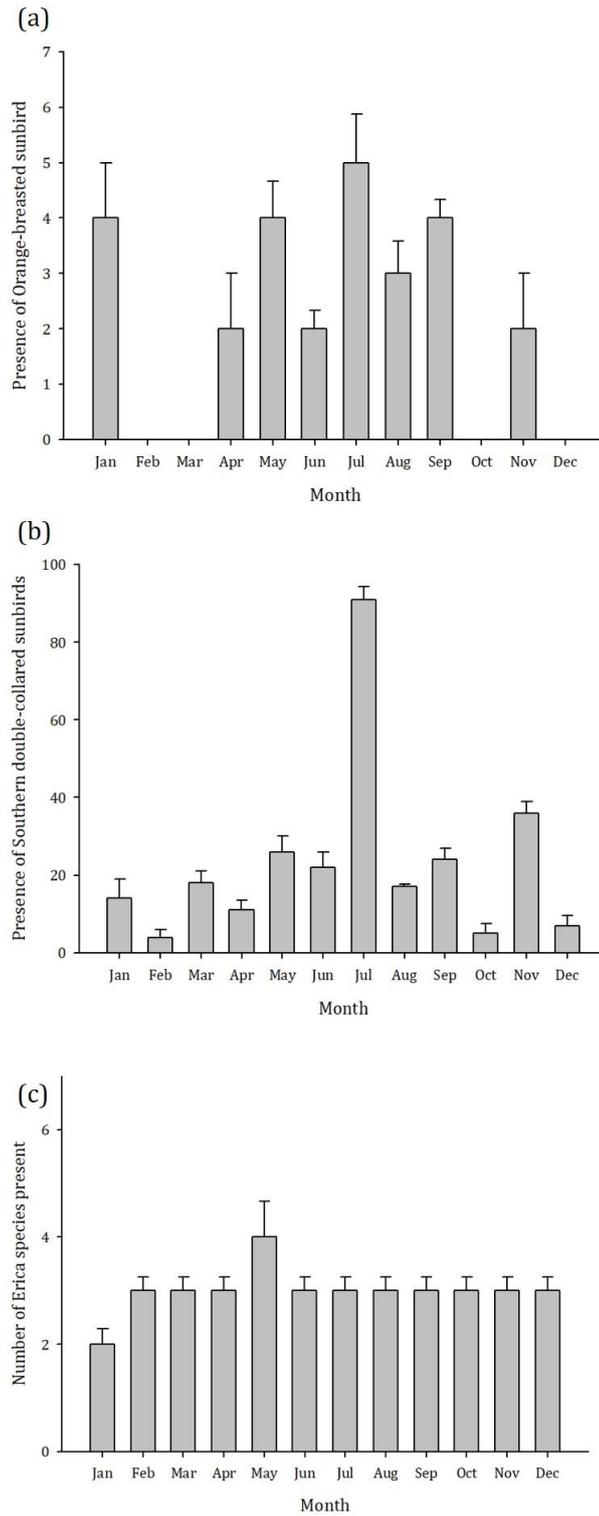
**Figure 2:** The presence of Cape Sugarbirds (black bars) and the number of protea species (grey bars) in flower for (a) 2014, May through to December, (b) 2015, January through to December and (c) 2016, January through to September.

The Orange-breasted sunbird (*Anthobaphes violacea*) was absent for the months of February, March, October and December and with a constant presence from April through to September (Fig. 3a). Four erica species (predominantly sunbird pollinated) occurred within the study area namely, *Erica densifolia* (Sticky red-and-green heath), *Erica discolor* (Two-colour heath), *Erica glandulosa sbsp fourcadei* (Fourcade's heath) and *Erica sessiliflora* (Green heath). Throughout the year the ericas were in flower with *E. discolor* and *E. glandulosa sbsp fourcadei* flowering for a longer period compared to *E. densifolia* and *E. sessiliflora*.

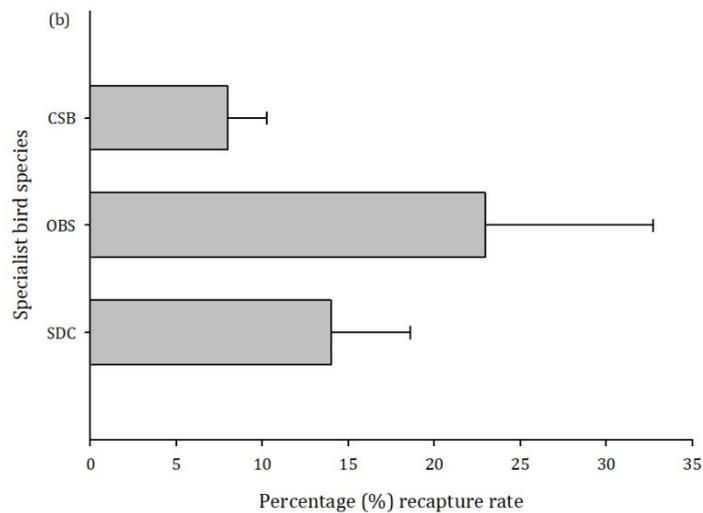
The Southern Double-collared sunbird (*Cinnyris chalybes*) was present throughout the year with a peak in numbers for July (Fig. 3b). Their presence in the area matched up with the presence of the erica species in the area (Fig. 3c).

The Amethyst (*Chalcomitra amethystina*), and Greater Double-collared (*Cinnyris afer*) sunbirds were caught fairly regularly with 26 and 16 records respectively. The Collared (*Hedydipna collaris*) and Malachite (*Nectarinia famosa*) sunbirds each had only one capture recorded within the study period. The Grey sunbird (*Cyanomitra veroxii*) had a capture record of four times.

Recapture data showed that of the three focus specialist bird species, the Cape sugarbird had the lowest percentage recapture rate over the two-year period, and the Orange-breasted sunbird the highest (Fig. 4)



**Figure 3:** The presence of (a) Orange-breasted sunbirds and (b) Southern double-collared sunbirds of the period of May 2014 to September 2016. (c) shows the number of erica species in flower. Error bars show standard error.



**Figure 4:** The percentage recapture rate for the three main specialist bird species over the three-year period. CSB = Cape sugarbird, OBS = Orange-breasted sunbird and SDC = Southern Double-collared sunbird. Error bars show standard error.

## Discussion

The two-year period in which flowering data was collected for both study sites have not shown any noticeable changes in flowering time based on personal observations and no noticeable changes in flowering months when combining the data into appendix 1 & 2. The method of using presence/absence data may not be the most effective or informative way of documenting this data, which may have an impact on the results. It may be better to look at making a count of individuals along a 1m band on either side of the trail. A growing concern for phenological processes is climate change effects. These data collected could form a baseline from which could be re-examined over the next few years. It has been suggested that climate change may disrupt flowering time and flight patterns of pollinators (Memmott *et al*, 2007). These shifts in flowering and pollinator presence would have a negative impact on important plant-pollinator interactions (Memmott *et al*, 2007; Chambers *et al*, 2013) which are important for floral communities to persist. It has already been seen in some temperate zones that there has been a shift of approximately 4 days per degree for first flowering and pollinator flight activity (Memmott *et al*, 2007). Not only would plant-pollinator systems be at risk, predator-prey interactions would be impacted too (Chambers *et al*, 2013).

The year-round presence of sunbirds (Fig. 3) within this section of fynbos gives a good indication that there are enough nectar producing (mainly *Erica* species) plants to sustain them in every month of the year. One should keep in mind that bird-pollination was not confirmed for all of the plant species considered, and their potential for bird-pollination was based flower morphology. The percentage recapture rate (Fig. 4) supports this for both the Southern Double-collared and Orange-breasted sunbirds, indicating high residency levels at the individual level, not just consistent species presence. *Erica discolor* seems to be present throughout the year acting as a permanent food source. The Southern Double-collared, Greater Double-collared, Amethyst and Orange-breasted sunbirds were all frequently observed throughout the year (Appendix 3 & 4), with the Grey, Collared and Malachite sunbirds less frequently. The reduced Collared sunbird presence is most likely because this is a forest species and not often seen in the fynbos (Sinclair & Ryan, 2009). The Grey sunbird is not exclusively a forest or fynbos species (Sinclair & Ryan, 2009) and is also more a winter visitor to the study region. The Malachite sunbird, although a fynbos species, may not be a common resident for this area which may explain its scarcity within the ringing data.

The Cape sugarbird (*Promerops cafer*) showed a presence in the area which matched the flowering of the four protea species, *L. cuneiforme*, *P. neriifolia*, *P. mundii* and *P. cynaroides*, which flower in the study area. Although there was presence of proteas towards the end of the year (October – December), this was the ending for the flowering period and flowers were not as abundant, which could explain the scarcity of sugarbirds for this period. This may also be indicative that the Cape sugarbird leaves the area in search of other food sources when the local proteas are not flowering. This is supported by the ringing data collectively over the two-year period. One of the sugarbirds ringed was recaptured on the other side of the Langkloof on a private nature reserve (Blue Hills Nature Reserve, 65km away direct line of flight) which had different protea species in flower at the time the local proteas had concluded their flowering. In addition, a low recapture rate of Cape sugarbirds suggests a more nomadic movement pattern with lower residency levels than the sunbirds.

The results show the area to have at least two endemic plant species as well as the presence of four of the six endemic fynbos bird species, namely the Orange-breasted sunbird, Cape sugarbird, Victorin's warbler (*Cryptillas victorina*) and Cape siskin

(*Crithagra totta*) (the warbler and siskin are present in the ringing data but were not necessary for this study), and are in themselves a reason to conserve the fynbos of this area. The movement of certain bird species from the area, like the Cape sugarbird, is an indication that food is a limiting resource and points to the importance of conserving the mosaic of fynbos patches outside the formal protected area network. This may also be an indication that large areas may be limiting and require conservation efforts to increase the size of the areas to be protected. Better research plans may need to be put in place to ensure conservation efforts will have an impact.

## **Conclusion**

This study has shown that this area of fynbos is an important part of the ecosystem which maintains plant-pollinator systems and acts as a food resource for several bird species. Sunbirds are sustained throughout the year with a source of nectar from a variety of plant species whereas the Cape sugarbird, although having a food source for most of the year, needs to source food elsewhere. As these data were not statistically analysed, any comparison which had been done were made with caution and the data should have further analysis using statistics.

Future studies should include monitoring the flowering times of the species over several years to determine if climate change might be affecting this flowering community. We only touched a small portion of the fynbos within this area, therefore further studies into the phenology of the Tsitsikamma National Park is needed to find and identify more plant species.

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**Appendix 1 – Plant species list for Kalander Kloof for presence and absence.**

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
1	<i>Agathosma capensis</i>	Aniseed buchu	Rutaceae							x	x	x	x		
2	<i>Agathosma ovata</i>	False buchu	Rutaceae			x	x		x			x			
3	<i>Alectra sessiliflora</i>	Verfblommetjie	Orobanchaceae			x	x		x			x			
4	<i>Aristea pusilla</i>		Iridaceae			x	x	x	x	x	x	x	x		
5	<i>Aspalathus alopecurus</i>		Fabaceae					x	x	x					
6	<i>Aspalathus chortophila</i>	Tea bush	Fabaceae		x	x	x	x	x	x					x
7	<i>Athanasia dentata</i>		Asteraceae				x	x	x						x
8	<i>Berzelia intermedia</i>	Knoppiesbos	Bruniaceae	x	x			x			x		x	x	x
9	<i>Bobartia aphylla</i>	Biesie	Iridaceae	x	x	x	x	x	x	x	x	x	x	x	
10	<i>Brunia noduliflora</i>	Fonteinbos	Bruniaceae		x			x							x
11	<i>Caesia contorta</i>	Blue grass lily	Hemerocallidaceae	x		x					x	x	x	x	x
12	<i>Carpobrotus deliciosus</i>	Sour fig	Aizoaceae							x	x	x			
13	<i>Cephalaria humilis</i>		Dipsacaceae	x				x	x	x	x				
14	<i>Ceratandra grandiflora</i>		Orchidaceae											x	x
15	<i>Chironia tetragona</i>		Gentianaceae	x	x	x	x	x							x
16	<i>Cliffortia ilicifolia var ilicifolia</i>	Rysbos	Rosaceae				x	x	x	x					
17	<i>Cliffortia stricta</i>		Rosaceae	x	x	x	x	x	x	x	x	x	x	x	
18	<i>Corymbium africanum subsp africanum</i>	Heuningbossie	Asteraceae												x
19	<i>Crassula sarmentosa var sarmentosa</i>		Crassulaceae						x	x					

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
20	<i>Disa hians</i>		Orchidaceae	x											x
21	<i>Disparago tortillis</i>		Asteraceae					x	x	x	x	x	x		
22	<i>Disperis capensis</i>	Moederkappie	Orchidaceae						x	x	x				
23	<i>Drosera aliciae</i>	Sundew	Droseraceae									x	x		
24	<i>Erica canaliculata</i>	Tree erica	Ericaceae	x						x					
25	<i>Erica copiosa var copiosa</i>		Ericaceae								x	x	x		
26	<i>Erica densifolia</i>	Sticky red-&-green heath	Ericaceae	x	x	x	x	x							x
27	<i>Erica discolor</i>	Two-colour heath	Ericaceae	x	x	x	x	x	x	x	x	x	x	x	x
28	<i>Erica formosa</i>	White heath	Ericaceae						x	x	x	x	x		
29	<i>Erica scabriuscula</i>		Ericaceae									x	x	x	
30	<i>Erica seriphiifolia</i>		Ericaceae	x	x	x	x								x
31	<i>Erica sessiliflora</i>	Green heath	Ericaceae					x	x	x	x	x	x	x	x
32	<i>Erica sparsa</i>		Ericaceae	x	x	x	x	x	x						x
33	<i>Erica triceps</i>		Ericaceae										x	x	
34	<i>Erica uberiflora</i>		Ericaceae	x	x	x	x	x							
35	<i>Euryops virgineus</i>	Rivierharpuis	Asteraceae						x	x	x	x	x		
36	<i>Gerbera serrata</i>		Asteraceae						x	x	x	x	x	x	x
37	<i>Gladiolus rogersii</i>	Riversdale blue bell	Iridaceae					x	x	x	x	x	x		
38	<i>Harveya purpurea</i>	Ink flower	Orobanchaceae										x	x	x
39	<i>Helichrysum cymosum subsp cymosum</i>		Asteraceae	x	x	x	x								x

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
40	<i>Helichrysum felinum</i>		Asteraceae							x	x	x	x	x	x
41	<i>Helichrysum petiolare</i>	Kooigoed	Asteraceae	x											x
42	<i>Helichrysum anomalum</i>		Asteraceae			x	x								
43	<i>Hippia frutescens</i>	Rankals	Asteraceae						x	x	x				
44	<i>Hypochaeris radicata</i>	Spotted Cat's ear	Asteraceae	x	x										
45	<i>Hypoxis hemerocallidea</i>	Star-flower	Hemerocallidea	x	x	x	x	x	x	x	x	x	x	x	x
46	<i>Indigofera brenidae</i>		Fabaceae								x	x			
47	<i>Indigofera erecta</i>		Fabaceae							x	x				
48	<i>Indigofera sulcata</i>		Fabaceae		x	x	x	x	x	x					
49	<i>Justicia capensis</i>	Money plant	Acanthaceae	x			x	x	x	x	x	x			
50	<i>Kniphofia uvaria</i>	Red-hot poker	Asphodelaceae					x	x	x	x				x
51	<i>Lanaria lanata</i>	Kapokblom	Lanariaceae	x										x	x
52	<i>Leucodendron eucalyptifolium</i>	Cone bush	Proteaceae	x	x	x	x	x	x	x	x	x	x	x	x
53	<i>Leucospermum cuneiforme</i>	Pin-cushion	Proteaceae								x	x	x	x	x
54	<i>Linum africanum</i>	African flax	Linaceae	x	x	x	x	x	x	x	x	x	x	x	
55	<i>Liparia hirsuta</i>	Common hard-leaf pea	Fabaceae						x	x	x	x	x		
56	<i>Lobelia flaccida</i>		Lobeliaceae	x	x	x	x							x	x
57	<i>Lobelia neglecta</i>	Wild lobelia	Lobeliaceae	x	x	x	x	x	x	x	x	x	x	x	x
58	<i>Lobelia tomentosa</i>		Lobeliaceae	x	x	x	x	x	x	x	x	x	x	x	x

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
59	<i>Metalasia muricata</i>	Blombos	Asteraceae	x	x	x	x	x	x	x	x	x	x	x	x
60	<i>Monopsis unidentata var unidentata</i>		Lobeliaceae	x	x	x	x						x	x	x
61	<i>Montinia caryophyllacea</i>	Wild clove-bush	Montiniaceae		x				x				x	x	
62	<i>Moraea brittaniae</i>		Iridaceae								x	x	x		
63	<i>Ostespermum moniliferum subsp moniliferum</i>	Bietou bush	Asteraceae						x	x	x	x	x		
64	<i>Othanna parviflora</i>	Bobbejaankool	Asteraceae								x	x	x		
65	<i>Oxalis incarnata</i>		Oxalidaceae						x					x	x
66	<i>Passerina falcifolia</i>	Outeniqua gonna	Thymelaeaceae									x	x	x	
67	<i>Pelargonium capitatum</i>	Rose-scented geranium	Geraniaceae						x	x	x	x	x	x	
68	<i>Pelargonium cordifolium</i>	Heart-leaved pelargonium	Geraniaceae	x						x	x	x	x	x	x
69	<i>Penaea cneorum subsp cneorum</i>		Penaeaceae	x	x	x	x	x	x	x	x	x	x	x	x
70	<i>Phylica sp</i>		Rhamnaceae	x									x	x	x
71	<i>Podalyria burchelli</i>	Hairy blossom-pea	Fabaceae		x	x	x	x	x	x	x	x			
72	<i>Polygala fruticosa</i>	Heart-leaf polygala	Polygalaceae								x	x	x	x	
73	<i>Polygala myrtifolia</i>	September bush	Polygalaceae	x					x	x	x	x	x	x	x
74	<i>Protea cynaroides</i>	King protea	Proteaceae									x	x	x	
75	<i>Protea mundii</i>	White forest sugarbush	Proteaceae	x	x	x	x	x	x	x					
76	<i>Protea neriifolia</i>	Bearded sugarbush	Proteaceae	x	x	x	x	x	x	x	x	x	x		
77	<i>Psoralea affinis</i>		Fabaceae								x	x			

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
78	<i>Relhania calycina subsp calycina</i>		Asteraceae	x					x	x	x	x	x	x	x
79	<i>Satyrium acuminatum</i>		Orchidaceae									x	x	x	
80	<i>Schistostephium umbellata</i>		Asteraceae							x	x	x	x		
81	<i>Senecio angulatus</i>	Cape ivy	Asteraceae					x	x						
82	<i>Senecio burchellii</i>	Ragwort	Asteraceae				x	x	x						
83	<i>Stachys aethiopica</i>	White salvia	Lamiaceae	x					x						
84	<i>Stoebe alopecuroides</i>	Katstertslangbos	Asteraceae						x	x	x	x			
85	<i>Struthiola hirsuta</i>		Thymelaeaceae							x	x				
86	<i>Sutera foetida</i>		Scrophulariaceae									x	x		
87	<i>Tritoniopsis caffra</i>	Snake flower	Iridaceae					x	x	x	x	x	x	x	
88	<i>Ursinia cf. anethoides</i>		Asteraceae	x				x	x	x	x	x	x		
89	<i>Virgillia divaricata</i>	Keurbooms	Fabaceae	x					x	x	x	x	x	x	x
90	<i>Wahlenbergia tenerrima var tenerrima</i>		Campanulaceae			x								x	x
91	<i>Watsonia fourcadei</i>	Suurkanol	Iridaceae									x	x	x	
92	<i>Watsonia knysnana</i>		Iridaceae	x	x	x	x	x							x

**Appendix 2 – Plant species list for Salt River for presence and absence.**

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
1	<i>Acalypha decumbens</i>	Brooms & brushes	Euphorbiaceae					x			x	x			
2	<i>Agapanthus praecox sbsp minimus</i>		Agapanthaceae	x	x	x									
3	<i>Agathosma ovata</i>	False buchu	Rutaceae		x	x	x	x	x	x	x	x	x		
4	<i>Albuca virens sbsp virens</i>	Bosui	Hyacinthaceae								x	x			
5	<i>Alepidia capensis</i>	Katazo	Apiaceae	x	x	x									
6	<i>Aptenia cordifolia</i>	Brakvygie	Aizoaceae	x										x	x
7	<i>Aristea pusilla</i>		Iridaceae	x	x			x	x	x	x	x	x		
8	<i>Aspalathus chortophilla</i>	Tea bush	Fabaceae	x	x	x	x	x	x	x	x				
9	<i>Athanasia dentata</i>		Asteraceae	x	x	x	x	x	x	x	x	x	x		
10	<i>Bonatea speciosa</i>	Green wood orchid	Orchidaceae							x	x	x	x		
11	<i>Bulbine lagopus</i>		Asphodelaceae								x	x	x	x	
12	<i>Burchellia bubalina</i>	Wild pomegranate	Rubiaceae									x	x	x	
13	<i>Caesia contorta</i>	Blue grass lily	Hemerocallidaceae	x	x						x	x	x	x	x
14	<i>Carissa bispinosa</i>	Num-num	Apocynaceae									x	x	x	
15	<i>Carpobrotus deliciosus</i>	Sour fig	Aizoaceae					x	x	x	x	x			
16	<i>Cephalaria humilis</i>		Dipsacaceae	x	x	x	x	x	x	x	x				
17	<i>Chaenostome cordatum</i>		Scrophulariaceae	x				x	x	x	x	x	x		
18	<i>Cliffortia stricta</i>		Rosaceae								x	x			
19	<i>Clutea laxa</i>		Euphorbiaceae								x	x			

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
20	<i>Commelina Africana var africana</i>	Yellow commelina	Commelinaceae	x			x	x							
21	<i>Cotyledon orbiculata subsp orbiculata</i>	Pig's ear	Crassulaceae							x	x	x	x		
22	<i>Crassula multicava sbsp multicava</i>	Skaduplakkie	Crassulaceae									x	x	x	
23	<i>Crassula orbicularis</i>	Klipblom	Crassulaceae							x	x	x	x	x	
24	<i>Crassula rubricaulis</i>		Crassulaceae			x	x	x							
25	<i>Crassula sarmentosa var sarmentosa</i>		Crassulaceae						x	x	x	x	x	x	
26	<i>Cyrtanthus elatus</i>	George lily	Amaryllidaceae	x	x										
27	<i>Delosperma patersoniae</i>		Aizoaceae	x						x	x	x	x	x	x
28	<i>Dietes iridoides</i>	Small forest iris	Iridaceae	x	x	x	x	x	x	x	x	x	x	x	
29	<i>Dipogon lignosus</i>	Cape sweet pea	Fabaceae								x	x	x	x	
30	<i>Erica canaliculata</i>		Ericaceae						x	x	x	x	x		
31	<i>Erica copiosa var copiosa</i>		Ericaceae								x	x			
32	<i>Erica discolor</i>	Two-colour heath	Ericaceae	x	x	x	x	x	x	x	x	x	x	x	x
33	<i>Erica glandulosa sbsp fourcadei</i>	Fourcade's heath	Ericaceae		x	x	x	x	x	x	x	x	x	x	
34	<i>Erica scabriuscula</i>		Ericaceae									x	x	x	
35	<i>Erica sparsa</i>		Ericaceae	x	x	x	x	x	x						
36	<i>Erica uberiflora</i>		Ericaceae			x	x	x							
37	<i>Eriocephalus africanus var africanus</i>	Wild rosemary	Asteraceae			x	x	x	x	x	x	x			
38	<i>Euryops virgineus</i>	Rivierharpuis	Asteraceae						x	x	x	x	x	x	

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
39	<i>Gerbera cordata</i>	Tongblaarblom	Asteraceae		x	x	x	x	x	x	x	x	x	x	
40	<i>Gladiolus gueinzii</i>		Iridaceae									x	x	x	
41	<i>Gladiolus rogersii</i>	Riversdale blue bell	Iridaceae								x	x			
42	<i>Habenaria arenaria</i>		Orchidaceae						x	x	x				
43	<i>Harveya purpurea</i>	Ink flower	Orobanchaceae											x	x
44	<i>Helichrysum cymosum var cymosum</i>		Asteraceae	x	x	x	x							x	x
45	<i>Helichrysum felinum</i>		Asteraceae											x	x
46	<i>Helichrysum petiolare</i>	Kooigoed	Asteraceae	x	x										x
47	<i>Hibiscus aethiopicus</i>	Dwarf wild hibiscus	Malvaceae	x	x	x									
48	<i>Hippia frutescens</i>	Rankals	Asteraceae	x						x	x	x			
49	<i>Holothrix parviflora</i>		Orchidaceae					x	x	x	x				
50	<i>Hypoxis hemerocallidea</i>	Star-flower	Hemerocallidaceae	x	x	x	x	x				x	x	x	x
51	<i>Indigofera stricta</i>		Fabaceae	x								x	x	x	x
52	<i>Indigofera sulcata</i>		Fabaceae		x	x	x								
53	<i>Isoglossa cillata</i>		Acanthaceae	x								x	x		
54	<i>Ixia orientalis</i>		Iridaceae									x	x		
55	<i>Kniphofia uvaria</i>	Red-hot poker	Asphodelaceae					x	x	x	x				
56	<i>Leucodendron eucalyptifolium</i>	Cone bush	Proteaceae	x	x	x	x	x	x	x	x	x			
57	<i>Leucospermum cuneiforme</i>	Pin-cushion	Proteaceae									x	x		

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
58	<i>Liparis remota</i>		Orchidaceae	x	x	x									
59	<i>Lobelia flaccida</i>		Lobeliaceae	x	x	x	x	x	x	x					
60	<i>Lobelia neglecta</i>		Lobeliaceae	x	x	x	x	x	x	x	x	x	x	x	
61	<i>Metalasia muricata</i>	Blombos	Asteraceae	x	x	x	x	x	x	x	x	x	x	x	
62	<i>Monopsis simplex</i>		Lobeliaceae	x									x	x	x
63	<i>Monopsis unidentata var unidentata</i>		Lobeliaceae	x	x	x	x	x	x	x				x	x
64	<i>Montinia caryophyllacea</i>	Wild clover-bush	Montiniaceae	x				x							
65	<i>Osteospermum monilifera sbsp monilifera</i>	Bietou bush	Asteraceae				x	x	x	x	x	x	x	x	
66	<i>Oxalis caprina</i>	Bokspootjie	Oxalidaceae				x	x							
67	<i>Oxalis incarnata</i>		Oxalidaceae										x	x	x
68	<i>Passerina falcifolia</i>	Outeniqua gonna	Thymelaeaceae										x	x	x
69	<i>Pelargonium capitatum</i>	Rose-scented	Geraniaceae		x	x	x						x	x	x
70	<i>Pelargonium cordifolium</i>	Heart-leaf pelargonium	Geraniaceae							x	x	x	x		
71	<i>Pelargonium zonale</i>	Horseshoe pelargonium	Geraniaceae											x	x
72	<i>Penaea cneorum var cneorum</i>		Penaeaceae		x	x	x	x	x	x	x	x	x	x	
73	<i>Phylica sp</i>		Rhamnaeae	x	x	x	x	x	x	x					
74	<i>Polygala fruticosa</i>	Heart-leaf polygala	Polygalaceae									x	x	x	x
75	<i>Polygala myrtifolia var myrtifolia</i>	September bush	Polygalaceae	x	x				x	x	x	x	x	x	
76	<i>Protea neriifolia</i>	Bearded sugarbush	Proteaceae	x	x	x	x	x	x	x	x	x	x		

#	Plant species	Common name	Family	J	F	M	A	M	J	J	A	S	O	N	D
77	<i>Rhynchosia caribea</i>		Fabaceae	x	x	x	x								
78	<i>Satyrium longicolle</i>		Orchidaceae										x	x	
79	<i>Schistostephium umbellata</i>		Asteraceae					x	x	x	x	x	x		
80	<i>Selago corymbosa</i>	Blombossie	Scrophulariaceae	x	x	x	x	x	x	x	x	x	x	x	x
81	<i>Senecio burchelli</i>	Ragwort	Asteraceae				x	x							
82	<i>Silene undulata var undulata</i>	Gunpowder plant	Caryophyllaceae									x	x	x	
83	<i>Stachys aethiopica</i>	White salvia	Lamiaceae	x	x	x	x	x	x	x	x	x	x	x	x
84	<i>Stoebe alopecuroides</i>	Katstertslangbos	Asteraceae							x	x				
85	<i>Streptocarpus rexii</i>	Twin sisters	Gesneriaceae	x	x	x	x	x						x	x
86	<i>Struthiola hirsuta</i>		Thymelaeaceae		x	x	x	x	x	x	x	x	x	x	
87	<i>Sutera foetida</i>		Scrophulariaceae	x				x	x	x	x	x	x	x	x
88	<i>Trifolium repens</i>	Dutch clover	Fabaceae	x								x	x	x	x
89	<i>Virgilia divaricata</i>	Keurbooms	Fabaceae	x	x					x	x	x			

**Appendix 3 – Bird species list for presence and absence.**

#	Bird species	Common name	J	F	M	A	M	J	J	A	S	O	N	D
1	<i>Anthobaphes violacea</i>	Orange-breasted sunbird	x	x	x	x		x	x	x	x		x	
2	<i>Chalcomitra amethystina</i>	Amethyst sunbird		x	x	x	x	x	x	x	x		x	x
3	<i>Cinnyris afer</i>	Greater Double-collared sunbird			x		x	x	x	x		x	x	
4	<i>Cinnyris chalybeus</i>	Southern Double-collared sunbird	x	x	x	x	x	x	x	x	x	x	x	x
5	<i>Cyanomitra veroxii</i>	Grey sunbird		x			x		x		x			
6	<i>Hedydipna collaris</i>	Collared sunbird				x								
7	<i>Nectarina famosa</i>	Malachite sunbird	x											
8	<i>Promerops cafer</i>	Cape sugarbird	x	x	x	x	x	x	x	x	x			

**Appendix 4 – Months bird-pollinated flowers were flowering and the presence of their potential pollinators**

<b>Month</b>	<b>Plant species</b>	<b>Probable pollinator(s)</b>
January	<i>Cyrtanthus elatus</i> <i>Erica densifolia</i> <i>Erica discolor</i> <i>Protea neriifolia</i> <i>Protea mundii</i> <i>Watsonia knysnana</i>	Southern Double-collared sunbird Malachite sunbird Cape sugarbird
February	<i>Cyrtanthus elatus</i> <i>Erica densifolia</i> <i>Erica discolor</i> <i>Erica glandulosa subsp fourcadei</i> <i>Protea neriifolia</i> <i>Protea mundii</i> <i>Watsonia knysnana</i>	Amethyst sunbird Southern Double-collared sunbird Grey sunbird Cape sugarbird
March	<i>Erica densifolia</i> <i>Erica discolor</i> <i>Erica glandulosa subsp fourcadei</i> <i>Protea neriifolia</i> <i>Protea mundii</i> <i>Watsonia knysnana</i>	Amethyst sunbird Greater Double-collared sunbird Southern Double-collared sunbird Cape sugarbird
April	<i>Erica densifolia</i> <i>Erica discolor</i> <i>Erica glandulosa subsp fourcadei</i> <i>Protea neriifolia</i> <i>Protea mundii</i> <i>Watsonia knysnana</i>	Amethyst sunbird Collared sunbird Southern Double-collared sunbird Cape Sugarbird
May	<i>Erica densifolia</i> <i>Erica discolor</i> <i>Erica glandulosa subsp fourcadei</i> <i>Erica sessiliflora</i> <i>Kniphofia uvaria</i> <i>Protea neriifolia</i> <i>Protea mundii</i> <i>Tritoniopsis caffra</i>	Amethyst sunbird Greater Double-collared sunbird Southern Double-collared sunbird Grey sunbird Cape sugarbird

	<i>Watsonia knysnana</i>	
June	<i>Erica discolor</i>	Orange-breasted sunbird
	<i>Erica glandulosa</i> subsp <i>fourcadei</i>	Amethyst sunbird
	<i>Erica sessiliflora</i>	Greater Double-collared sunbird
	<i>Kniphofia uvaria</i>	Southern Double-collared sunbird
	<i>Protea neriifolia</i>	Cape sugarbird
	<i>Protea mundii</i>	
	<i>Tritoniopsis caffra</i>	
July	<i>Cotyledon orbiculata</i> subsp <i>orbiculata</i>	Orange-breasted sunbird
	<i>Erica discolor</i>	Amethyst sunbird
	<i>Erica glandulosa</i> subsp <i>fourcadei</i>	Greater Double-collared sunbird
	<i>Erica sessiliflora</i>	Southern Double-collared sunbird
	<i>Kniphofia uvaria</i>	Grey sunbird
	<i>Leucospermum cuneiforme</i>	Cape sugarbird
	<i>Protea neriifolia</i>	
	<i>Protea mundii</i>	
<i>Tritoniopsis caffra</i>		
August	<i>Cotyledon orbiculata</i> subsp <i>orbiculata</i>	Orange-breasted sunbird
	<i>Erica discolor</i>	Amethyst sunbird
	<i>Erica glandulosa</i> subsp <i>fourcadei</i>	Greater Double-collared sunbird
	<i>Erica sessiliflora</i>	Southern Double-collared sunbird
	<i>Kniphofia uvaria</i>	Cape sugarbird
	<i>Leucospermum cuneiforme</i>	
	<i>Protea neriifolia</i>	
<i>Tritoniopsis caffra</i>		
September	<i>Burchellia bubalina</i>	Orange-breasted sunbird
	<i>Cotyledon orbiculata</i> subsp <i>orbiculata</i>	Amethyst sunbird
	<i>Erica discolor</i>	Southern Double-collared
	<i>Erica glandulosa</i> subsp <i>fourcadei</i>	Grey sunbird
	<i>Erica sessiliflora</i>	Cape sugarbird
	<i>Leucospermum cuneiforme</i>	
	<i>Protea cynaroides</i>	
	<i>Protea neriifolia</i>	
	<i>Tritoniopsis caffra</i>	
<i>Watsonia fourcadei</i>		
October	<i>Burchellia bubalina</i>	Greater Double-collared
	<i>Cotyledon orbiculata</i> subsp <i>orbiculata</i>	Southern Double-collared sunbird
	<i>Erica discolor</i>	

*Erica glandulosa* subsp *fourcadei*  
*Erica sessiliflora*  
*Leucospermum cuneiforme*  
*Protea cynaroides*  
*Protea neriifolia*  
*Tritoniopsis caffra*  
*Watsonia fourcadei*

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November	<i>Burchellia bubalina</i> <i>Erica discolor</i> <i>Erica glandulosa</i> subsp <i>fourcadei</i> <i>Erica sessiliflora</i> <i>Leucospermum cuniforme</i> <i>Protea cynaroides</i> <i>Tritoniopsis caffra</i> <i>Watsonia fourcadei</i>	Orange-breasted sunbird Amethyst sunbird Greater Double-collared sunbird Southern Double-collared sunbird
December	<i>Erica densifolia</i> <i>Erica discolor</i> <i>Erica sessiliflora</i> <i>Leucospermum cuniforme</i> <i>Watsonia knysnana</i>	Amethyst sunbird Southern Double-collared sunbird

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## **Chapter 6 - Conclusion**

### **Overview**

The fynbos is a geographically restricted biome of South Africa with exceptional levels of biodiversity and endemism. There have been many studies on the plant communities of this biome and the animal interactions found within it, however there is still more that has yet to be discovered and understood. Some parts of the Western and Eastern Cape have received plenty of attention whereas certain areas have received little to no research. This MSc degree served as an opportunity to explore an area of the Tsitsikamma that is rarely studied, allowing for research to be done on pollination systems, reproductive success in patchily distributed plant populations and phenology work.

### **Contribution to biological and ecological knowledge**

I collected data on two plant species which have not received much attention, especially in the southern Cape of South Africa. Data collected have helped contribute to the knowledge of the pollination systems of *Kniphofia uvaria* and *Chasmanthe aethiopica*, helping in the confirmation that they are reliant on birds for pollination. This contributes to other studies which have found other species of *Kniphofia* (Brown *et al*, 2009; Brown *et al*, 2010; Brown *et al*, 2011) to be bird-pollinated, and contributed to more information on the pollination system of *Chasmanthe*.

Although there has been phenology data collected for the fynbos of the southern Cape, most has occurred in the Cape St Francis area (Cowling, 1983; Pierce & Cowling, 1991; Pierce & Moll, 1994). No studies could be found for the fynbos occurring within the Tsitsikamma section of the Garden Route National Park. Thus, this study has contributed more to the knowledge of the phenology of southern Cape fynbos. This study has also contributed data comparing the presence of important bird pollinator species to the flowering of the plant species which they pollinate.

### **Functional role of birds**

The selective exclusion studies conducted on *K. uvaria* and *C. aethiopica* showed that birds are important for the pollination of these species. Although the caged treatments for *K. uvaria* produced some fruit, seed set, and the number of potentially viable seeds was much lower compared to plants that allowed for bird visitation. This shows that birds

likely play an important role in the pollination of this species and that it has a bird-pollination breeding system. The pollination system for *C. aethiopica*, based on the data collected, showed strong evidence for bird-pollination. The caged treatment had very little viable seed produced giving an indication that insects are not successful pollinators.

The results obtained when testing for the presence of the Allee effect in these two populations of plants species showed that, regardless of the size of patches or distance between patches, pollination seems to be successful for *C. aethiopica*, however *K. uvaria* population may exhibit an Allee effect. This gives yet another indication that birds are important to have in this area as they aid even the smallest patches of bird-pollinated plants with pollination. It may be worthwhile to do statistical tests using seeds per flower to combine data from both fruit and seed set, enabling a more holistic measure of fecundity to be directly measured between patches of different sizes.

The year-round presence of nectar feeding bird species and nectar producing plants in this section of fynbos gives an indication that plant-bird interactions are important for this plant community. Sunbirds play a crucial role in the pollination of a number of the plant species which were identified.

### **Summary statement**

In summary, birds play a key role in the pollination system of *K. uvaria* (Asphodelaceae) and *C. aethiopica* (Iridaceae), with their removal from the system likely to lead to the loss of these plant species.

The results of the phenology and mist netting showed that there are endemic nectar feeding bird species making use of this patch of fynbos, namely the Orange-breasted sunbird and Cape sugarbird. There is a high likelihood that a number of the plant species rely on birds for pollination with the birds relying on these plant species as a food resource. This gives a valid reason for the importance of conserving not only this fynbos community but also fynbos found on both private and governmentally owned land. This statement can be further supported with evidence from the mist netting that birds move from patch to patch in search of food (the Cape sugarbird a prime example).

With regards to the main questions addressed in this thesis, the studies done have shown (1) evidence for a bird-pollination breeding system for *K. uvaria* and *C. aethiopica*, (2) Allee effect is not likely present in these two plant species, (3) phenology of the fynbos in

the area differs slightly to the fynbos in the winter rainfall area, (4) flowering times of bird-pollinated plants coincidence with the presence of the birds which pollinate them.

### **Suggestions for future research**

This thesis had a few shortcomings which should be considered for any future studies on *K. uvaria* and *C. aethiopica*. There were no tests done for self-incompatibility where the seeds from fruits produced for the bagged treatment were planted to see if they would germinate. Effectiveness of a single pollinator visit, and supplemental hand-pollination studies have been done for *C. aethiopica*, but studies are needed for *K. uvaria*. Trapping birds visiting *K. uvaria* and *C. aethiopica* inflorescences were not done to study the pollen loads of the birds. There were no direct tests done for the effects of patch size on the visitation rates of bird-pollinators for both plants species. It could be useful to do more general year-round network type studies of birds and plants in this local community to determine which species are linked ecologically.

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