

Inbreeding Depression and Ovule Pre-emption
in *Bulbine bulbosa* (R. Br.) Haw.
(Asphodelaceae)

Kathleen Janet Owen

B.A.; Grad. Dip. Nat. Res.; BSc. (Hons), University of New England

A thesis submitted for the degree of Doctor of Philosophy
of the University of New England
July 2007

University of New England
Armidale, NSW 2351, Australia

This thesis is dedicated to

Neville Owen Walker. 1928 - 2006

Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.



Kathleen Janet Owen

Acknowledgments

I would like to thank my supervisors Dr. Glenda Vaughton and Assoc. Prof. N. Prakash. To Prakash, for his embryological expertise and assistance during the histological part of my project - I just made it - before his retirement! Thankyou to Glenda for her expert academic advice throughout my candidature and critiques of the draft versions of this thesis. Thanks also to Mike Ramsey for statistical advice. I also appreciate the general support and friendliness of the Academic staff in Botany. Thankyou particularly to, David Backhouse and Caroline Gross for stepping in as supervisors during Glenda's study leave.

I am grateful for the assistance of an Australian Post Graduate Award in addition to the financial support of The School of Environmental Sciences & Natural Resources Management, UNE.

Thankyou to Warren and Gloria Sheather who allowed me to work on their property - providing me with a beautiful and peaceful field site.

Thanks to our Botany technicians, Richard, Ian and Doug for your help and advice on all things technical. Thankyou to Geraldine and Sandy for help in administration.

Liisa - Thankyou for your editing skills and also your friendship, motivation and encouragement and the chats about teenagers!

Thankyou to my cohort of post grads (some now Drs!) Dr. Kerri, Azadeh, Dr. Mohammad, Dr. Kirsten, Dr. Boyd, Ian T., Dr. Lachlan, Dr. Xiufu, Dr. Karen, Sarah, Dr. Robert, Dalvinder and Siamak. I have really enjoyed your company over the years.

To my best friends Cath, Chez, Haz, and Kerri (in alphabetical order) besides just being flat-out great, inspirational, supportive and fun to be with - you're my best buddies and each of you bring something special and unique to my life.

Lastly, but of course not least - my family – Thank you goes to my children Clancy, Nelsyn and Thomas. You're my life! Thank you to my Aunty, Joan Walker - I could not have done it without you! To my sisters, Linda and Carolyn. To Nelsyn, Trish and Chez for looking after me on the home stretch! To Eric for child swapping!

Abstract

This thesis examines the causes of self-sterility and consequences of inbreeding on seed set and progeny fitness in *Bulbine bulbosa* (Asphodelaceae). I conducted my research utilising both glasshouse plants and natural populations of *B. bulbosa*.

I investigated the causes of reduced self-fertility. Two mechanisms can be responsible for self-infertility in hermaphroditic flowers: physiological self-incompatibility and inbreeding depression. I conducted a histological investigation of ovule development after self- and cross-pollination, up to 7-days post-pollination. Examination of cleared specimens at 2-days post-pollination indicated that the mechanism reducing self-fertility was post-zygotic because double fertilisation was apparent after both self- and cross-pollination. This eliminated gametophytic and sporophytic self-incompatibility. Examinations of ovule development at 5- and 7-days post-pollination indicated that abortion was significantly higher after self- compared to cross-pollination. There was no indication that the arrest of selfed ovule development occurred at a single stage, eliminating a late-acting physiological self-incompatibility mechanism. The evidence indicated that reduced self-fertility was probably due to early-acting inbreeding depression.

I investigated the effects of inbreeding on progeny fitness after selfing and mating between related individuals. The fitness of inbred progeny was assessed at ovule fertilisation, seed set, seed mass, seed germination, seedling growth and survival, days to first flower, number of inflorescences and flowers, and ovule and pollen production. After complete selfing and biparental inbreeding, inbreeding depression reduced progeny fitness at all life-cycle stages except ovule fertilisation, seed mass and, percent and speed of seed germination. Cumulative uniparental and

biparental inbreeding depression was 0.99 and 0.74 respectively, indicating it was unlikely inbred progeny would survive to reproductive maturity.

I also examined mating between plants found in close proximity.. Close proximity matings resulted in reduced seed set compared to mating between individuals at further distances. Individuals found in close proximity are likely to be related and mating between these individuals probably represents biparental inbreeding

I examined pollen limitation and self pollen interference. Natural seed set was pollen limited in three flowering seasons. The quantity of pollen deposition exceeded the number of ovules 5-fold in each year. Open seed set was less than cross seed set yet fertilisation rates were similar, indicating that natural seed set was limited by the quality, but not the quantity, of pollen deposited onto the stigmas. Supplementing open pollinated plants with cross pollen did not increase seed set, because ovules had already been fertilised by poor quality pollen. In a pollen chase experiment, the application of cross pollen after self pollen also failed to increase seed set compared to self-pollination, indicating that self-pollen tubes interfere with cross pollen by pre-empting ovules.

I conclude that inbreeding facilitated by both selfing and mating between related individuals is costly to *B. bulbosa* populations. Inbreeding results in inbreeding depression causing ovules to abort reducing natural seed set. Consequently, these ovules are wasted and are unavailable for outcrossing. Inbreeding reduces fecundity and potentially recruitment to successive generations. Finally, inbreeding depression is a major selective force maintaining a predominately outcrossing mating system in *B. bulbosa*.

Table of Contents

CHAPTER 1.....	1
GENERAL INTRODUCTION.....	1
<i>Study species</i>	7
<i>Thesis aim</i>	11
<i>Chapter aims</i>	11
CHAPTER 2.....	14
THE MECHANISM OF SELF-INFERTILITY IN <i>BULBINE BULBOSA</i>: SELF- INCOMPATIBILITY OR INBREEDING DEPRESSION?.....	14
INTRODUCTION	14
<i>Aim</i>	20
METHODS	20
<i>Plant material</i>	20
<i>Pollinations</i>	21
<i>Clearing</i>	22
<i>Sectioning and staining</i>	23
<i>Statistical analysis</i>	23
RESULTS	24
<i>Mature ovules</i>	24
<i>Fertilised ovules 2-days PP</i>	25
<i>Developing ovules 5- and 7-days PP</i>	25
<i>Unfertilised ovules</i>	26
<i>Aborting ovules</i>	26
<i>Undeveloped ovules</i>	27

<i>Undetermined ovules</i>	27
<i>Fertilisation frequencies</i>	37
<i>Ovule development at 5- and 7-days post-pollination</i>	38
<i>Undetermined ovules</i>	38
DISCUSSION.....	41
CHAPTER 3	45
UNIPARENTAL AND BIPARENTAL INBREEDING DEPRESSION IN <i>BULBINE BULBOSA</i> (ASPHODELACEAE)	45
INTRODUCTION	45
<i>Aim</i>	49
METHODS	50
<i>Experimental design – hand pollinations</i>	49
<i>Ovule fertilisation, seed abortion and seed set</i>	52
<i>Later life-cycle stages</i>	52
<i>Seed mass</i>	52
<i>Seed germination</i>	53
<i>Seedling survival, leaf length and biomass</i>	53
<i>Flowering</i>	54
<i>Pollen and ovule production</i>	54
<i>Relative performance and inbreeding depression</i>	55
<i>Statistical analysis</i>	57
RESULTS	58
<i>Seed production</i>	58
<i>Progeny fitness</i>	58
<i>Relative performance and inbreeding depression</i>	68
<i>Inbreeding depression</i>	68
DISCUSSION	73

CHAPTER 4.....	79
THE EFFECT OF DISTANCE BETWEEN MATES ON SEED PRODUCTION IN <i>BULBINE</i>	
<i>BULBOSA</i> (ASPHODELACEAE).....	79
INTRODUCTION.....	79
<i>Aim</i>	82
METHODS.....	82
<i>Experimental plants</i>	82
<i>Experimental design – hand pollinations</i>	83
<i>Ovule fertilisation, seed abortion and seed set</i>	84
<i>Statistical analysis</i>	84
RESULTS.....	85
<i>Seed set</i>	85
<i>Seed abortion</i>	85
<i>Ovule fertilisation</i>	85
DISCUSSION.....	88
CHAPTER 5.....	93
POOR QUALITY POLLEN INTERFERENCE AND POLLEN LIMITATION IN <i>BULBINE</i>	
<i>BULBOSA</i> (ASPHODELACEAE).....	93
INTRODUCTION.....	93
<i>Aim</i>	96
METHODS.....	97
<i>Study site</i>	97
<i>Pollen deposition</i>	98
<i>Field experiment</i>	98
<i>Natural selfing rates</i>	101
<i>Inbreeding depression</i>	102

<i>Pollen interference</i>	103
RESULTS	107
<i>Pollen deposition</i>	107
<i>Field experiment</i>	107
<i>Natural selfing rates</i>	111
<i>Inbreeding depression</i>	111
<i>Pollen interference</i>	111
DISCUSSION	115
CHAPTER 6	121
GENERAL DISCUSSION AND CONCLUSIONS	121
<i>Early-acting inbreeding depression controls self-fertility</i>	121
<i>Pollen interference causes ovule wastage and ovule discounting</i>	124
<i>Inbreeding depression</i>	127
BIBLIOGRAPHY	133