THE EFFECTS OF POPULATION SIZE ON *AGATHOSMA COLLINA* (RUTACEAE) AND ITS CONSERVATION IMPLICATIONS

BY

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Contents

Abstract

Agathosma collina is a relatively typical widespread, outcrossing shrub species, endemic to the coastal vegetation between the Agulhas Plain and Heidelberg in the Southern Cape. Although not a rare species, its endemism makes it a suitable candidate for a study of population size effects and the conservation implications for similar outbreeding species.

Theory predicts that we should expect demographic problems to arise, for ecological or genetic reasons, when population size becomes too small. Initially, therefore, a study was carried out to assess if there was any discernible effect of population size on the population structure. As predicted by theory, the demographic status of small populations was worse than larger ones, measured in terms of seedling recruitment with small populations having relatively fewer seedlings than large. This effect was quite small and far from dramatic.

Demographic aspects such as length of flowering, predation and seed set per capsule in different sized populations were studied. Inflorescence predation rates were found to increase to a peak during the peak flowering time of *A. collina* and medium sized populations showed the highest predation rate while lower predation percentages in large and small populations were probably a result of predator satiation (large populations) and crypsis (small populations). Seed set per capsule varied from mostly one seed per capsule in small and medium populations to two seeds per capsule in large populations in 1988. However, 1989 data showed an almost identical spread in the numbers of seeds per capsule in the different sized populations.

None of the above results seemed sufficiently consistent over time or population size to be of importance regarding population structure differences between different sized populations.

In order to obtain an indication of the amount of genetic exchange between populations, gene flow studies were carried out using pollen dispersal and seed dispersal. Although direct estimates of gene flow proved difficult to obtain, these studies indicated that gene flow was limited. Neighbourhood area resulting from estimates of pollen dispersal distances was only $2.12m^2$ compared to ballistic seed dispersal neighbourhood area of $3.05m^2$, while that resulting from possible myrmecochorous seed dispersal was $41.97m^2$. Neighbourhood size proved to be heavily dependent on population density and was very specific to a population and care should be taken when extrapolating results to different populations or plant species.

Cross pollination experiments were carried out to attempt to identify an optimal outbreeding distance. Although pollen from 7km away produced the highest capsule set of all the treatments this could have resulted from heterosis. The species is self compatible but geitonogamous matings produced lower capsule set than outcrossed matings. Of the pollination distances which could be expected under natural circumstances the nearest neighbour crosses produced the most capsules. This corroborates the results of the gene flow studies which indicate that gene flow may be over very short distances.

Inbreeding depression in small populations of *A. collina* seems likely since neighbourhood size is very small resulting in a low probability of pollen transfer from any great distance and since *A. collina* is self compatible allowing geitonogamous matings in very small populations

Electrophoresis was then attempted to confirm the above results from a genetic aspect. Unfortunately this was not successful.

This study provided some empirical support for theoretical predictions of the effects of population size on plants but suggests that the main problems may be genetic rather than ecological. The magnitude of the population size effects were small and then only in very small populations and are therefore probably only of concern under rare circumstances.

CHAPTER 1

INTRODUCTION

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1. INTRODUCTION

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A. PROBLEMS IN SPECIES CONSERVATION

1. INTRODUCTION

The natural vegetation of South Africa is under tremendous pressure. Of the original approximately 7 million ha of the fynbos biome only approximately 4.7 million ha remained in 1986 (Jarman 1986). On the outskirts of every town and city new housing developments are going up and agricultural land is continually encroaching on naturally vegetated areas. Large areas of natural vegetation are becoming increasingly difficult to find. The best land for conservation is frequently good for development and conservation is the cause that loses out. Remnant patches are often all that remain of once vast areas of natural vegetation. Lowland fynbos vegetation (Moll et al 1984), for example, has been reduced from an estimated original area of 2850800ha to 895500ha (31%) (Jarman 1986).

Our natural areas are, therefore, being reduced in size and our conservation efforts are relying increasingly on nature reserves. It is now seldom possible to create reserves large enough to conserve all habitats and species types and as more and more land is disturbed, we have to begin to rely on smaller, well placed reserves for the preservation of smaller suites of species (Soulé and Simberloff 1986).

As the area available for conservation becomes less and less, conservationists will more often face the problem of determining just how little habitat a species can have and yet survive (Shaffer 1987). Small reserves are more vulnerable than large ones in many ways. Edge effects, for example, would have a much greater influence on small reserves than large ones. Hall and Veldhuis (1985) identified invasive alien plants, agriculture and presumed genetic decline in critically small populations as some of the major hazards facing threatened species.

Other problems which arise from this tendency towards smaller reserve size are those of extinctions, deterministic or stochastic, to which small populations are particularly susceptible.

A fixed trend causing decline of a species, such as uncontrolled alien invasion of a reserve, would be an example of deterministic extinction.

Shaffer (1981) describes four types of uncertainty a population may be subjected to, which could lead to extinction:

1. Demographic stochasticity - random events in the survival and reproduction of individuals. After a fire in a small population of *Leucadendron macowanii*, a rare, dioecious species found only in a few localities, only male seedlings may emerge.
2. Environmental stochasticity - Variation in births and deaths owing to changing environmental conditions such as competition and predation
3. Natural catastrophies - random environmental perturbations, for instance drought, may lead to the local extinction of a species.
4. Genetic stochasticity - genetic drift may lead to the fixation of a less fit genotype in a population.

The dilemma with which we are now faced is which of the four above is the most important in the long and short term and how they affect management planning.

In the short term, the effects of random demographic factors should take precedence in the management of a population in order to prevent its immediate extinction (Gilpin and Soulé 1986, Lande and Barrowclough 1987, Lande 1988 and Ewens *et al* 1987). In the population of *L. macowanii*, mentioned above, female plants would perhaps have to be introduced into the population. Once the short term survival of the population has been ensured, however, genetic processes become important in the conservation of populations and their long term evolution, especially in small populations (Gilpin and Soulé 1986. Lacy 1988, Lande and Barrowclough 1987).

Shaffer (1981) emphasises the importance of environmental stochasticity in larger populations which might override any genetic or demographic considerations.

All these along with natural stochasticity are important but natural stochasticity is not easy to predict and the most obvious way to counteract it would be to maintain separate populations of a species, in geographically different areas, so that if one population is destroyed by some natural event then the other may survive.

It can be seen, therefore, that there are several factors which could lead to local or global extinction of a population over different spatial and temporal scales. Lande (1988) highlights the need for an understanding of the interaction of demographic and genetic factors in the extinction of small populations.

2. BROAD RESEARCH MOTIVATION

Basic theory predicts area dependent extinctions, i.e.: the larger the area the fewer extinctions that can be expected. This study aims to investigate the genetic and demographic effects of small population size in the field and to get a direct estimate of these effects so as to compare them to theoretical predictions.

Little work has been done on plant breeding systems in South Africa (Steiner 1987), and plant genetics has until recently been neglected as a research topic (Hall 1987). There is a distinct lack of information on non-proteaceous fynbos shrub biology and data on the demographic effects of small population size are lacking for most fynbos species. In their survey of fynbos islands within the Knysna forest, however, Bond et al. (1988) found "little evidence for a strong link between population size and extinction" amongst the species they studied but attributed species loss from these islands rather to changes in disturbance patterns. With the fragmentation that occurs daily in the fynbos vegetation, as a result of human expansion, this topic has become very important in fynbos conservation.

That genetic variation must be preserved in order to increase the probability of both the short- and long-term survival of species (Allendorf and Leary 1986) is without doubt. However, the extent of genetic variation that must be conserved creates a problem.

This project, therefore, tests some of the demographic and genetic effects involved in conservation, an understanding of which is necessary in the management and recovery of small and endangered populations (Ellstrand and Elam 1993, Grant et al 1988, Lacy 1988, Lande 1988).

To develop management strategies for conservation and regeneration of some or all of the elements of a particular plant population, the complex relationships between pollinators and plants must be investigated and understood (Whitehead *et al* 1987) and the results of research in gene flow and other aspects of genetic biogeography are likely to be of practical importance to conservation (Hall 1987). This study was one of the first direct tests of the effects of population size in a metapopulation of a fynbos species and was intended to contribute to the small amount of knowledge of the breeding systems of fynbos plant species and will assist in the answering of some of the questions raised.

B. SPECIES CONSERVATION - THEORETICAL CONSIDERATIONS

3. MINIMUM VIABLE POPULATION SIZE (MVP)

There is growing interest in minimum viable population size in population biology. A minimum viable population size (MVP) is an estimate of the minimum number of organisms of a particular species that constitutes a viable population (Boyce 1992) or can be defined as the minimum population size below which a population of a species will not survive for any given length of time. This is an important concept in that it can be used as a guideline in the formation of nature reserves.

Soulé (1987) argues that although the term minimum viable population size may sound self defeating, in that it can be seen to be advocating a small nature reserve size, the concept is useful in providing a minimum area necessary to preserve a species, if there is absolutely no other choice. A larger area, however, is the ideal situation and the greater the genetic heterogeneity the species can maintain the greater the chance of survival of the species.

The effects of environmental and natural stochasticity are unpredictable and may be very large in a restricted population. It may be better, therefore, to maintain at least two populations, the main aim being to protect them against demographic and genetic stochasticity and to an extent against environmental and natural stochasticity. The main protection then, would be the preservation of two or more populations isolated from each other.

Combined genetic and demographic studies of plant species should, therefore, revolve around the concept of population size. They would provide information on the population size required to minimise inbreeding and genetic drift and to maximise genetic diversity allowing for the evolution of a species over time (Soulé 1980, Boecklen 1986) and assist in isolating demographic problems (e.g. lack of pollinators and competition) which may arise as a result of small population size.

4. THE GENETIC ASPECT

4.1. Heterozygosity And Fitness

An increase in heterozygosity within a population will generally lead to an increase in fitness within the population (Usher 1987). This could be as a result of the masking of recessive deleterious alleles when two unrelated individuals breed or as a result of heterozygote advantage.

Since two unrelated individuals are unlikely to carry the same deleterious alleles, when they breed, the recessive deleterious alleles from the one parent are masked by the dominant normally functioning allele from the other parent (Ledig 1986, Richards 1986). If, however, two closely related individuals breed, there is a higher chance of getting a homozygous recessive genotype causing a reduction in fitness. Maintenance of heterozygosity in a population would, therefore, increase the fitness of that population.

Under severe environmental conditions the advantages of heterozygosity become clear (Beardmore 1983). In a genetically heterogeneous population a detrimental stochastic environmental event may destroy a certain genotype within it. By the very fact that the population is heterogeneous, though, some of the individuals will be better suited to the survival of that event than others and will survive to reconstruct the population. If, on the other hand, the population was homogeneous for a specific trait, which reduced fitness with respect to the destructive event, then there would be a higher likelihood of local extinction.

To ensure long term survival of a population it is essential to preserve its genetic diversity in order to buffer the actions of such stochastic perturbations (Lesica et al 1988, Gilpin and Soulé 1986).

4.2. Maintenance And Loss Of Genetic Variation

4.2.1. Inbreeding

Inbreeding, whether due to self fertilization or cross pollination between genetically similar parent plants, leads to homozygosity (Ralls *et al* 1986, Hall 1987, Drury 1974, Antonovics and Ellstrand 1984) and the reduction of genetic variability in the population (Drury 1974, Usher 1987).

Populations that gradually become inbred will probably be less severely affected by the increased homozygosity than a population that is rapidly reduced in size and is forced into immediate inbreeding (Simberloff 1988, Beardmore 1983, Frankel and Soulé 1981). Inbreeding exposes deleterious alleles which with very slow inbreeding and selection may be eradicated from the population without too much damage being caused (Simberloff 1988). A normally outbreeding population which is suddenly subjected to strong inbreeding pressures, however, will rapidly lose heterozygosity and deleterious alleles may be exposed at higher levels than the species can tolerate, thus causing loss of fitness. The loss of fitness due to inbreeding is called inbreeding depression.

Inbreeding is important when designing a small reserve for a species. An effective method of protecting a population may be to encourage subdivision allowing the subpopulations to become homozygous within themselves. Boecklen (1986) using a computer simulation found that heterozygosity in a population was in fact increased with division of the population into subunits.

It is generally agreed that genotypic variation among individuals in populations provides a starting point for evolutionary change (Schlising and Turpin 1971). In the long term, therefore, reduced heterozygosity will also limit the potential for the future evolution of a species (Ginsberg 1987) which is essential for its survival through the gradual environmental fluctuations which occur on an evolutionary time scale.

A workshop on population genetics (1987) held in Pietermaritzburg expressed concern that inbreeding depression was often overemphasised in conservation biology. In fact many plants are self pollinating (autogamous). Cleistogamous plants exist. This simple fact emphasises the problems in generalising the deleterious effects of inbreeding.

4.2.2. Genetic Drift

The stochastic fluctuations due to sampling or loss of parental genes in a population are known as genetic drift (Usher 1987, Richards 1986, Frankel and Soulé 1981). In a large population random selection of alleles goes on all the time but since the population is large it is unlikely that an allele will be lost altogether. If an allele is lost in one mating it may not be lost in another. If, however, the population is small, many genes will be rare simply because of the size of the population. Random sampling in a small population can lead to the chance loss of an allele. Genetic drift may eventually lead to the fixation of deleterious genes, a reduction in fitness and increased probability of extinction of the population.

Most species of seed plants are composed of multiple isolated or semi-isolated breeding units each of which may adapt to local environmental conditions (Levin and Kerster 1974)). If each breeding unit is a sub-population of a metapopulation, then the possible scenario here is that of a series of different small populations each homozygous within themselves but all the populations when taken as a whole are a highly heterozygous mixture. Subdivided populations may rapidly lose variation from within each subpopulation but they will retain variation across subpopulations (Lacy 1987) resulting in the minimising of the loss of genetic drift of the metapopulation.

Genetic drift, therefore, could be a problem for the conservation of a population which is subdivided. Rates of genetic drift in subdivided populations are influenced by inter-population migration rates (Boecklen 1986) and drift, therefore, can be countered by the occasional gene transfer between subpopulations (Lacy 1987).

4.3. Local Adaptation

Populations or sub-populations of a species from different areas may become locally adapted to their environment. Crosses between individuals of different populations may then show outbreeding depression (Waser and Price 1989). Outbreeding depression has traditionally been reported only from crosses between isolated populations of single species or from even wider crosses (Waser and Price 1983).

Waser and Price (1983) found that if *Ipomopsis aggregata* (Polemoniaceae) pollen was transported from a plant beyond some optimal distance from the female then the seed set was depressed. The optimal pollen transfer distance was very short, between 1 and 100m only, indicating that outbreeding depression may result over very short distances.

4.3.1. Population Structure

In a divided, patchy population gene flow may be limited resulting in genetic differentiation of subpopulations (Pickett and White 1985). The population incorporating all these subpopulations (demes) is called a metapopulation. Gene movements by both pollen and seed are determinants of the size of a genetic neighbourhood (Schaal 1980) which is a sub-unit of a metapopulation within which there is panmixia. In a population of individuals continuously distributed throughout a two dimensional range the isolation by distance model of Wright (1946) can be used to estimate genetic neighbourhood size.

The isolation by distance model proposes a neighbourhood size of $4\pi d\sigma^2$ where σ^2 is the variance of the dispersal distances and d is the density of the population. Neighbourhood size is, therefore, defined as equivalent to the number of reproducing individuals in a circle whose radius is equivalent to twice the standard deviation of the gene dispersal distance (Levin and Kerster 1974, Golenberg 1987).

Depending on the dispersal mechanism used by the plants for their pollen and seed, the neighbourhood size will differ. With an estimation of neighbourhood size and therefore subpopulation structure a better idea can be developed with respect to homozygosity and genetic drift in small populations.

Gene flow distances may be short and the relatedness of plants within populations is likely to be inversely related to distance (Levin 1984). Patches of related individuals would, therefore, occur in a population (Hamrick 1982) and a genetically structured population would arise.

5. DEMOGRAPHY

The demographic component of this study with respect to population size must not be overlooked. If the population size becomes too small do changes occur within the population? Pollinators need sufficient reward for visiting a bush or vegetation patch to make it worth their while. If a patch is small and isolated from any other, the energy required for an insect to reach it may be more than the insect would gain by visiting it and so it will visit another larger, less isolated patch instead. As a result pollen transfer within small populations may be reduced. This would lead to a reduced seed set and a decline in the fitness of the population as a whole.

What the above paragraph sets out is one manifestation of the Allee effect described by Lande (1988). In terms of pollinator attraction the small population may no longer be effective and may have reached a threshold number of individuals at which it is no longer viable and the steady decline in numbers will continue.

The same might be true of dispersers, with small populations having fewer creatures to disperse their seeds for instance. With a decrease in myrmecochorous seed production the presence of myrmecochorous ants in the population may become reduced resulting in increased predation on exposed seeds (Blommaert 1972).

If smaller populations of a species have reduced reproductive ability then they would be much more vulnerable to extinction than larger populations. Total extinction may not occur as a result of one disaster which killed all the plants in the population, but the population, which may already be reduced in number as a result of a small seed store, will be more vulnerable to the disturbance than a healthy population.

C. PROJECT DESCRIPTION

6. INTRODUCTION TO A. COLLINA

Rutaceae, a family common in the fynbos biome, has 103 species listed in the S.A. Red Data Book for the fynbos and karoo biomes (Hall and Veldhuis 1985) and of these 46 belong to the genus *Agathosma*. *A. collina*, although not listed in the Red Data Book, is endemic to the coastal vegetation between the Agulhas plain and Heidelberg. Although fairly common in this region its endemism makes *A. collina* a suitable candidate for this study. Outcrossing widely distributed species, such as *A. collina*, are theoretically likely to be more sensitive to genetic loss through inbreeding in small populations than naturally inbreeding species so the species being studied probably represents an extreme case of the anticipated effects.

A. collina is a dense, aromatic shrub to +1,5m tall occurring on stabilised dunes (Pillans 1950). The white flowers are borne on terminal clusters (12 to 20 flowers per cluster) and the ovary has three carpels (Pillans 1950).

Andromonoecy, which is known to occur in 65 *Agathosma* species (Steiner 1987), is a breeding system in which male and hermaphrodite flowers occur on the same plant. This has the effect of increasing pollen/ovule ratios (P/O) (Cruden 1977). Increased P/O in turn, is hypothesised to increases the male competitiveness within a population (Stephenson and Bertin 1983, Steiner 1987). *A. collina* is both andromonoecious and protandrous and the species is insect pollinated.

The leaves are yellow green and as a result the plant is easily spotted in the field as it stands out clearly among the other plants in the community.

Seed dispersal in this species is first due to ballistic ejection of the seed from the capsule on the shrub after which ants disperse the myrmecochorous seeds further.

7. KEY QUESTIONS

I studied several aspects of the population biology of *A. collina* which may influence its response to population fragmentation. I wished to explore empirical evidence for the hypothesised effect of population size and isolation in this relatively common species. If this species showed marked responses to fragmentation as predicted by theory, one could use theory and the information gained from this species for threatened taxa with similar attributes.

The following questions were addressed:

1. What is the breeding system of *A. collina*?
2. How does population size affect demography?
3. Is there a difference in reproductive output in different sized populations?
4. What is the gene flow pattern within and between populations of the species?
5. Is there an optimal distance from which pollen should come which would increase seed set over pollen from any other distance?
6. Is there a difference in genetic diversity in different sized populations?

8. STUDY SITE AND CLIMATE

The study area is in the Fresh Water Sands Private Nature Reserve between Quoin point and Agulhas on the Southern Cape Coast. The area forms part of a unique hummock-blowout dune topography found nowhere else on the subcontinent's coastline (Tinley 1985).

The vegetation of the study site has been classified as Dune Asteraceous Fynbos which has a high cover of non-ericaceous ericoids and lacks proteoids and which is confined to deep, well-drained calcareous sands (Cowling et al 1988).

The area has not been subjected to unnatural disturbance for at least 20 years, before which it was subjected to light grazing. This area provides a study site in conditions very similar to those which may have occurred in pristine systems which would be difficult to find elsewhere in the Western Cape.

The area is characterised by cold wet winters and hot dry summers (Figures 1.1 and 1.2). The predominant winds in the area are north-west in winter and south-east in summer (Figure 1.3). The windiest season of the year (December-January) with an average wind speed of 24km/h coincides with the summer dry season (Tinley 1985) making these wind blasted areas some of the driest on the Agulhas plain (Cowling et al 1988).

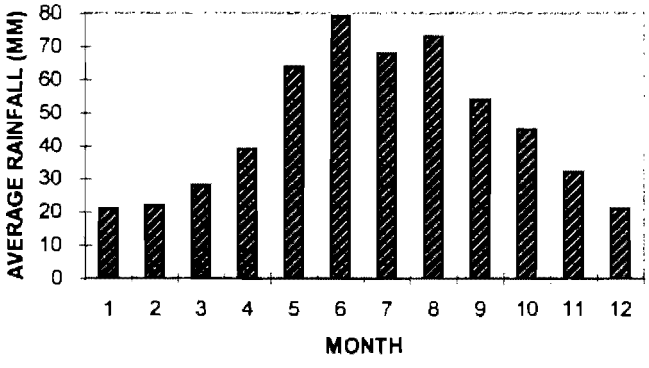

Figure 1.1 Average monthly rainfall at Agulhas (1882-1984) (measured in mm)

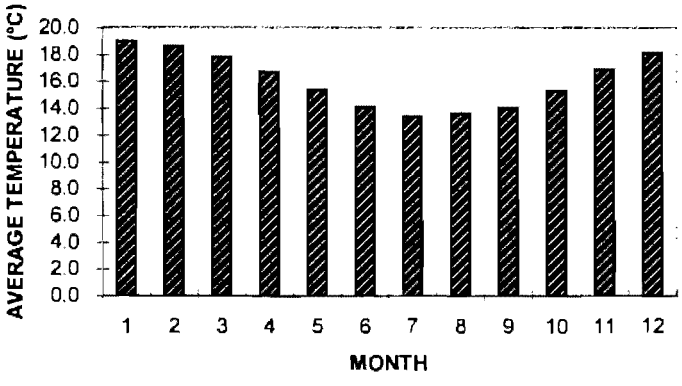

Figure 1.2 Average daily air temperature at Agulhas (1920-1984) (measured in °C)

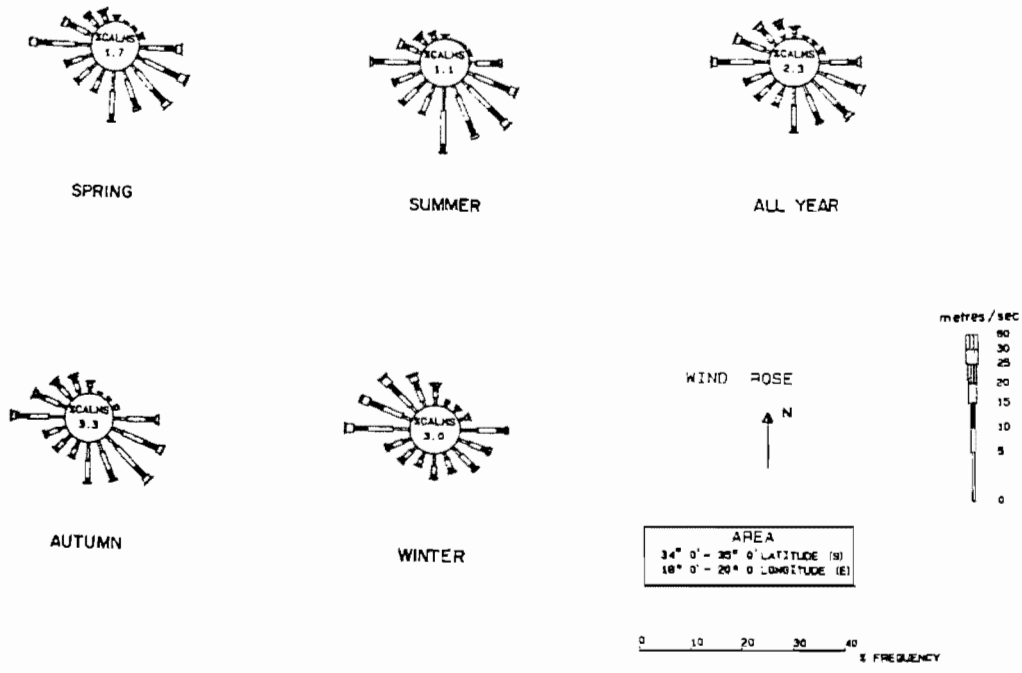

Figure 1.3 Wind roses for the Agulhas coastline (From De Decker 1989).

CHAPTER 2

SAMPLING DESIGN AND PHENOLOGY OF AGATHOSMA COLLINA

1. THE SAMPLING OF POPULATIONS AND INDIVIDUALS

2. DESCRIPTION OF THE FLOWER PHENOLOGY OF A. COLLINA

2.1. Method

2.2. Results And Discussion

1. THE SAMPLING OF POPULATIONS AND INDIVIDUALS

Populations of *A. collina* were chosen within the Fresh Water Sands Private Nature Reserve. The criteria used to choose the populations were, that the populations should be distinct from other populations and that they should be of different sizes. Ease of access to the populations also played a role since they would be visited often throughout the study period.

Eleven populations were sampled ranging in size from two to approximately 1000 individuals (Table 2.1). Only individuals which were large enough to flower were counted. In each population up to 30 individuals were marked and used throughout the study. In populations with fewer than 30 individuals all plants were marked. Of the 30 individuals chosen in each population 10 were small plants (canopy area $<0,12m^2$), 10 were medium sized plants (canopy area $0,12\text{-}0,75m^2$) and 10 were large plants (canopy area $>0,75m^2$). Individuals of the different size classes were chosen at random and marked with a number so that a particular individual could be studied over the whole study period. These populations and the marked plants in them were used throughout the study.

The study area is a natural metapopulation of *A. collina* consisting of sub-populations of various sizes and isolation. There are three possible causes of this fragmentation into sub-populations. Firstly, they may have been separated by dune blowouts subdividing a once continuous population. This would have been an ongoing process over a long period of time (Tinley 1985) and in this case the populations may have been isolated for long periods.

Secondly, the area was subjected to some degree of burning before it was protected. This burning may have caused the local extinction of some populations leading to fragmentation of a few large populations into a series of smaller ones. Blow-out areas would have increased after burns since the ground would have been laid bare which would have helped to increase the rate of fragmentation of the populations.

Finally long distance dispersal events may have allowed seed to germinate and plants to become established away from the original populations forming a new population.

Since all the populations occur in the same area they were therefore subjected to the same environmental pressures.

Table 2.1 The different population numbers and their sizes.

POP NO.	SIZE
12	1000
1	800
2	200
10	90
7	75
5	70
3	60
4	40
6	30
14B	13
8	11
14	2

2. DESCRIPTION OF THE FLOWER PHENOLOGY OF A. COLLINA

In the previous chapter a broad phenological outline of *A. collina* has been described. For an in depth study such as this, however, further functional detail and background is necessary for a complete understanding of the plant reproductive processes.

In self-compatible plants, self pollination is often limited either by temporal or physical separation of the mature anther and stigma or by some combination of the two (Moore and Lewis 1965). Since the flowers of *A. collina* are protandrous in that the anthers dehisce before the stigmas become receptive, prevention of self pollination in this instance is via a temporal mechanism. Further observation also revealed that the flowers of each inflorescence were synchronous with respect to the times at which they are staminate or pistillate (a combination of temporal and spatial separation) but neighbouring inflorescences may be at different stages. This would prevent cross pollination of flowers on the same

inflorescence thus increasing the chances of outcrossing between individuals of the species. Self pollination is however possible by geitonogamous crosses between inflorescences on the same plant.

In order to carry out pollination studies it is essential to get an estimate of the time taken for an inflorescence to develop from a bud through the pistillate and staminate stages, to the stage at which the stigmas were no longer receptive and flowering was completed. To achieve this it was necessary to monitor individual flowers.

2.1. Method

The development of the flower was divided into four stages each ending at the beginning of the following stage. The first stage began when the petals of "mature" buds were beginning to unfurl, the second began when the flowers were fully open with mature anthers just prior to anthesis, the third stage began while the anthers were still presenting pollen but the styles were beginning to elongate so that the stigmas could be seen to be appearing and the fourth stage began when the stigmas were receptive (the stigmas became damp and light brown in colour).

Sixteen flowers were monitored, four sets of four being monitored from each of four different stages. Four plants were studied with each individual providing four flowers, one at each of the different stages. The stages were used in order to reduce the time needed to get the sequence of events involved in the development of the inflorescence.

2.2. Results and Discussion

The following is the sequence of events deduced from this study:

Day 1:	Petals beginning to unfurl.
Day 2:	One half to all flowers open no stamens appearing.
Day 3:	One half of the stamens extended.
Day 4:	All anthers extended and beginning to dehisce.
Day 5:	Anthers all dehisced and pollen is presented.
Day 6:	Anthers still presenting pollen but styles beginning to grow.
Day 7:	Anthers no longer presenting pollen and beginning to wilt.
Day 8:	Styles fully extended.
Day 9:	Stigmas receptive (stigma damp and light brown in colour).
Day 10:	Stigmas receptive.
Day 11:	Stigmas less receptive (drying out and becoming darker in colour)
Day 12:	Stigmas no longer receptive, petals wilting.

From the above it can be seen that the anthers presented their pollen, and the stigmas were receptive for approximately three days while the time taken from the bud stage until the stigmas were receptive was approximately 8 days. The time taken from the first petals unfolding to the time when the petals began wilting was twelve days. For hand pollination experiments this is essential information in order to minimise damage to the bagged inflorescence caused by the removal of the bags to monitor the progress of the inflorescences until they could be pollinated.

This study indicated that if inflorescences were bagged when the first flowers were beginning to open then the hand pollinations could be carried out approximately eight days later.

CHAPTER 3

FLORAL BIOLOGY OF A. COLLINA

1. INTRODUCTION

Andromonoecy, the breeding system in which male and hermaphrodite flowers occur on the same plant is known to occur in 65 *Agathosma* species (Steiner 1987). Andromonoecy results in an increase in the pollen/ovule ratios (P/O's) (Cruden 1977), improving the chances of successful pollination (Stephenson and Bertin 1983, Steiner 1987).

A possible explanation for the evolution of andromonoecy put forward by Willson (1983) is that the production of extra male flowers might increase the conspicuousness of the floral display (at lower cost than producing hermaphrodite flowers) and thus might increase the attractiveness of the plant to pollinators. Augspurger (1980) found that the major pollinator of *Hybanthus prunifolius* was attracted consistently only to high densities of floral resources, and that the level of attraction was dependent upon the floral density at both the population and individual level.

A. collina has a long flowering season. I tested the prediction that when the number of inflorescences on a plant are at their lowest, the proportion of male flowers in an inflorescence would be high in order to increase the attractiveness of the inflorescence without incurring the cost of producing hermaphrodite flowers. During peak flowering season, when the floral display is maximal, the attraction of the plant to pollinators would in any case be high and hermaphrodite flowers would be more favoured.

The aim of this part of the study, therefore, was to see if there were any changes in the number of flowers on inflorescences or in the male:hermaphrodite ratios of *A. collina* over the period of build up to the optimal flowering time.

2. METHODS

2.1. Flower Season

The number of inflorescences with any open flowers on each of 30 marked individuals in each of the marked populations was estimated at least monthly but every two weeks in the flowering season. This made it possible to get an estimate of the peak flowering season for *A. collina*. In some of the populations as described in chapter 2 there were fewer than 30 flowering individuals and so all the individuals were monitored. Once there were more than 200 inflorescences per individual it became impractical to estimate differences in inflorescence number on each bush. Any individual with more than 200 inflorescences was designated as such.

This data was then graphed in order to illustrate peak flowering time of A. collina.

2.2. Seasonal Changes In Floral Sex Ratios

Inflorescences were collected from individuals in two populations (5 and 7). Population 5 consisted of 70 individuals and population 7 consisted of 75 individuals. Five individuals in pop 5 and four in pop 7 were sampled. The inflorescences collected were the ones which were most conspicuous, i.e. those with the greatest number of flowers. In most cases five inflorescences were collected from each individual but at times this was not possible. Where five inflorescences were not found all the flowers on the plant were collected. Collections were made monthly from June through to November 1990. The number of male and hermaphrodite flowers on each inflorescence were counted as well as any that showed signs of predation (see chapter 5 for predation results). Those which were indistinguishable as male or hermaphrodite due to damage such as that caused by predation or which were still in the bud stage, were ignored. The only flowers used in the analysis of M/H ratios were those clearly distinguishable as male or hermaphrodite.

3. RESULTS:

3.1. Flower Season

In June the number of inflorescences on *A. collina* plants were at their lowest. Peak flowering occurred around October/November (Figure 3.1). Figure 3.1 is just one of the graphs that could be generated from the data collected but it is typical of all the individuals sampled. (See chapter 5 for further discussion on length of flowering)

It was observed that inflorescences were present on the species being studied throughout the year and that *A. collina* is andromonoecious within an inflorescence.

3.2. Seasonal Changes In Floral Sex Ratios

The number of flowers per inflorescence in populations 5 and 7 was low outside the main flowering time and varied from one in June to more than 30 in the flowering season. The average number of flowers per inflorescence showed a steady increase from July (10.1) through to October (18.2) (Figure 3.2). In July, the average number of flowers per inflorescence was significantly lower than in the September to November flowering period (Table 1), with October having a significantly higher number of flowers per inflorescence than the June to September period.

Table 3.1 One way ANOVA of the monthly differences in the number of flowers per inflorescence on *A. collina* plants.

Sources	SS	d.f.	F-Ratio	Sig. Level
Between Months	1595,03	5	9,04	0,000
Within Months	7234,83	205		
Total	8829,86	210		

The monthly M/H ratios are given in figure 3.3. In June the M/H's were significantly higher than the other months (Table 2). The M/H ratio in July, although not significantly different from August to November, is the highest of the July-November period. From August, just prior to peak flowering season, the M/H ratios are much reduced and this remains so through to October/November which is the optimum flowering period.

Table 3.2 One way ANOVA of the monthly differences in the M/H ratios of inflorescences on *A. collina* plants.

Sources	SS	d.f.	F-Ratio	Sig. Level
Between Months	410.82	5	10.36	0,000
Within Months	1579.03	199		
Total	1989.85	204		

The results show, therefore, that with an increasing number of inflorescences with open flowers on a plant the average number of flowers per inflorescence also increases. As the peak flowering time approaches, the inflorescences become larger and more plentiful. Before the beginning of the increase in the number of inflorescences, however, the M/H ratio drops from nearly four males per hermaphrodite flower to 0,5 or less (Figure 3.3).

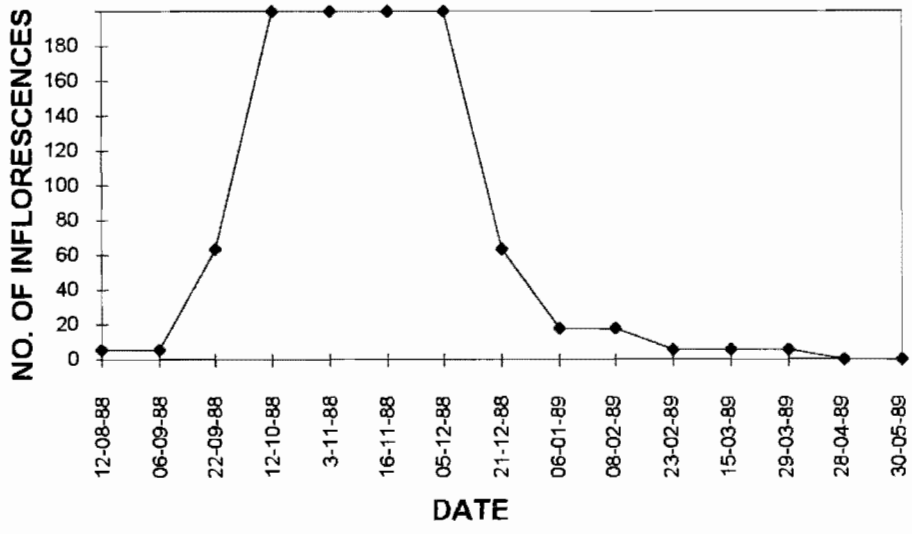

Figure 3.1. The number of inflorescences with open flowers on an individual in population 1 over a period of 250 days including the peak flowering time

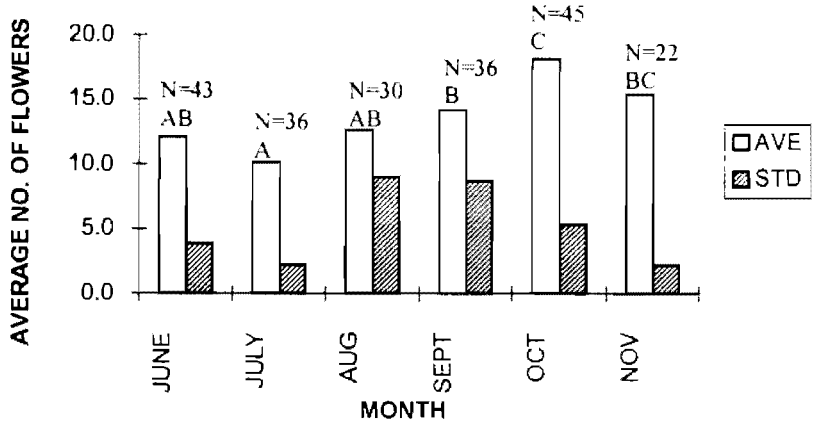

Figure 3.2. The average number of flowers per inflorescence on *A. collina* measured monthly from June to November in populations 5 and 7. The relationships, in terms of significant differences are indicated by the capital letters on the top of each bar. Bars with the same capital letters indicate no statistically significant differences (ANOVA).

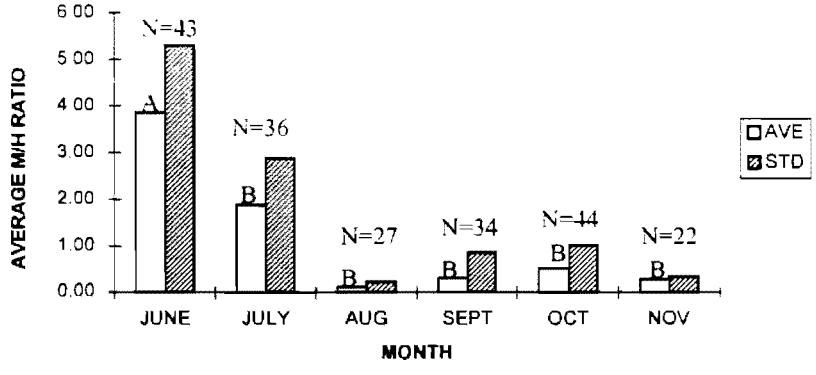

Figure 3.3. Average monthly male:hermaphrodite ratios of inflorescences of *A.collina*. Bars with the same capital letters indicate no statistically significant differences (ANOVA)

4. DISCUSSION

When an individual has only a few flowers, it loses its attractiveness to pollinators (Augspurger 1980). A method of increasing the attractiveness of an inflorescence would be to increase the amount of pollen available to pollen collecting insects (Primack and Lloyd 1980). In June and July, while the number of inflorescences on *A.collina* plants was low, the male to hermaphrodite ratios on the inflorescences were higher than at peak flowering time

thus maintaining the visual attractiveness of the inflorescence and maintaining high levels of pollen availability. Primack and Lloyd (1980) found that the shrub *Leptospermum scoparium* used less resources in the production of male flowers than in the production of hermaphrodite flowers. Andromonoecy in *A. collina*, therefore, is possibly a way of increasing the conspicuousness of the floral display (Willson 1983) as well as increasing the amount of pollen available (Primack and Lloyd 1980) but at low cost.

If on the other hand the attractiveness of an *A. collina* plant was high, due to large numbers of inflorescences with a large number of flowers, as is the case during peak flowering time, then the number of pollinator visitations would be high. It would then be cost effective for the plant to have an increased number of hermaphrodite flowers in the inflorescence (Figure 3.3), since the chances of successful pollination would increase and more resources could be made available for seed production. An increased amount of pollinators would lead to more successful pollinations and thus high P/O's would not be as necessary.

Further evidence, such as a study of seasonal seed set variation would assist in the further assessment of the hypothesis presented.

5. CONCLUSION

A. collina displays a mass flowering phenomenon as described by Augspurger (1980). At peak flowering time the large number of inflorescences on an individual would attract sufficient pollinators to reduce the need for an excessive supply of pollen to ensure pollination. The plant puts more energy into the development of hermaphrodite flowers, thus increasing the seed set potential of the individuals.

The results show that with an increasing number of inflorescences on an *A. collina* plant there is a decrease in the male/hermaphrodite ratio within the inflorescences on a plant.

CHAPTER 4

POPULATION SIZE AND ITS INFLUENCE ON POPULATION STRUCTURE

1. INTRODUCTION
2. METHOD
3. RESULTS
4. DISCUSSION

1. INTRODUCTION

The central theme in this thesis is the implication of numbers in the survival of populations of a species. Are small populations more prone to extinction than large populations? The aim of this chapter was to study the effect of different sized populations of *A. collina* on population size structures.

A. collina is killed by fire and regenerates from seed. Reseeding species whose seeds are stimulated to germinate by fire would be expected to have even aged populations dating from the last fire. In *A. collina*, however, recruitment occurs between fires and populations consequently have mixed age and therefore size structures. The assumption was therefore made that plant size is related to age of the plant. Although size is possibly a poor estimate of age it gives some indication of age class distributions in a population.

If population size influences population growth rate, as predicted by the theory, then the size structures of populations should differ with proportionally fewer juveniles in small populations and the most in large populations.

2. METHOD

In 10 of the marked populations (pops 12, 2, 5, 6, 7, 8, 10, 3, 4, 14B) at least one circular or semicircular plot of 10m in diameter was sampled. In the large populations 3 such plots were sampled while in the medium and small populations 2 plots and 1 plot were sampled respectively.

In populations 5, 6 and 7 full circle plots were studied while in the other populations, excluding population 8, semicircular plots were studied. In population 8, a small population consisting of 11 plants, the whole population area was sampled since this population had a very low *A. collina* density and the individuals were well spread out.

The ground cover directly below points every 10cm along each circle diameter was noted (ie. at 100 points) in order to obtain an estimate of vegetation cover in each population. In each plot the canopy area of every *A. collina* plant was measured and used to study the population structure, the percentage *A. collina* cover and the mean *A. collina* plant size.

Seedlings were defined for this study as those individuals having a canopy area of $<0.001m^2$.

The density of *A. collina* plants and total density of all species in the plots mentioned above was also calculated from the data collected.

3. RESULTS

All size class distributions in the plots throughout the populations were very similar (Figures 4.1, 4.2 and 4.3). Size class distributions were generally bimodal with relatively high percentages of seedlings, few plants in intermediate size classes, and, in most cases, a second peak at plant sizes between $0.1m^2$ and $1m^2$. There were however plots throughout the different sized populations which did not have this peak of adult plants.

There was a positive relationship between the population size and the percentage of *A. collina* plants which were seedlings in each plot ($p<0.05$, $r = 0.47$) (Figure 4.4)

Regression analysis showed no relationship between the average *A. collina* size in a population and the percentage seedlings in each plot (Figure 4.5). Population density in terms of *A. collina* cover and total vegetation cover also showed no relationship to the percentage *A. collina* seedlings in each plot when compared by regression analysis (Figures 4.6 and 4.7).

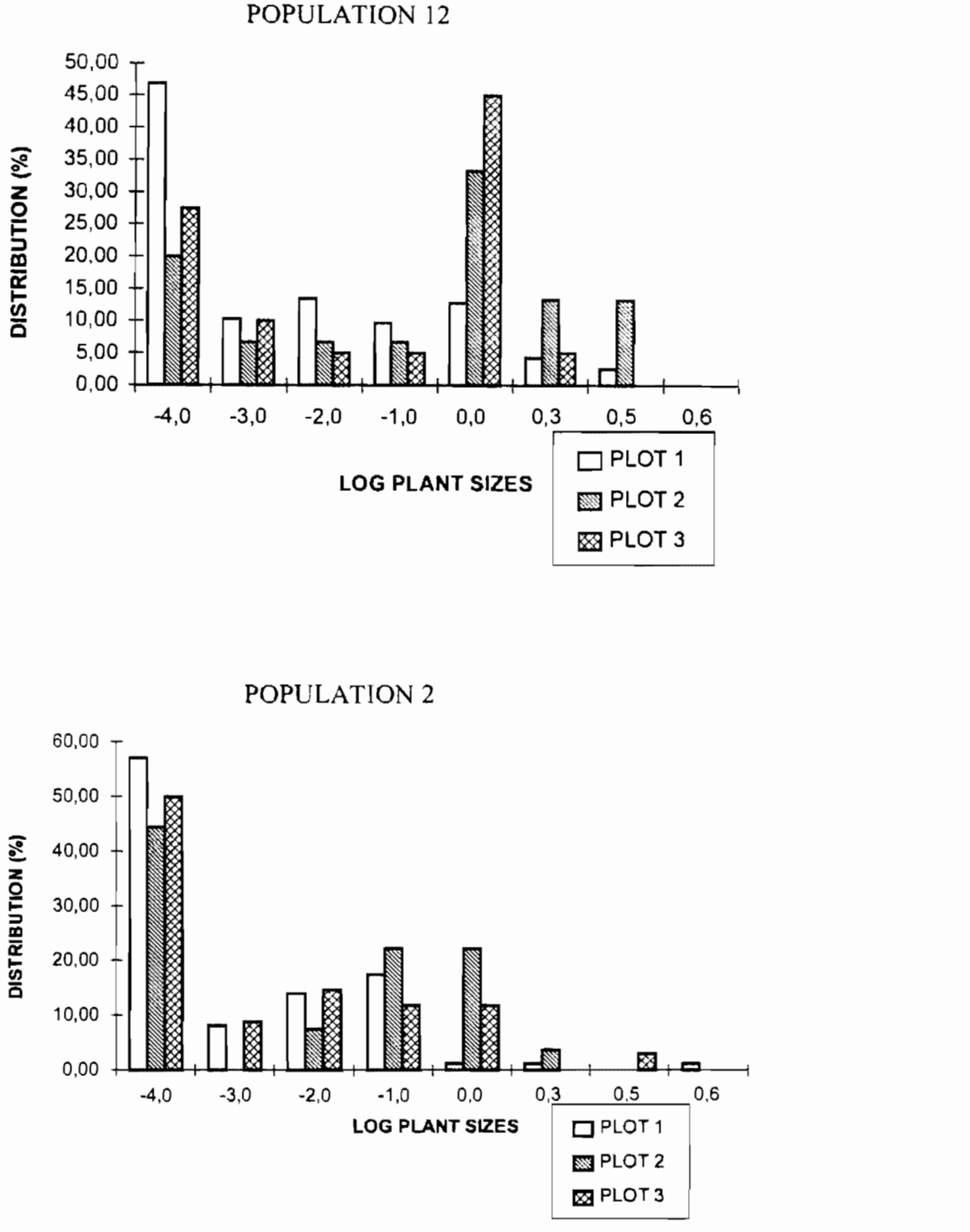

Figure 4.1. Size class distributions of the two large populations sampled (populations 12 and 2). Three replicate plots were studied in each population.

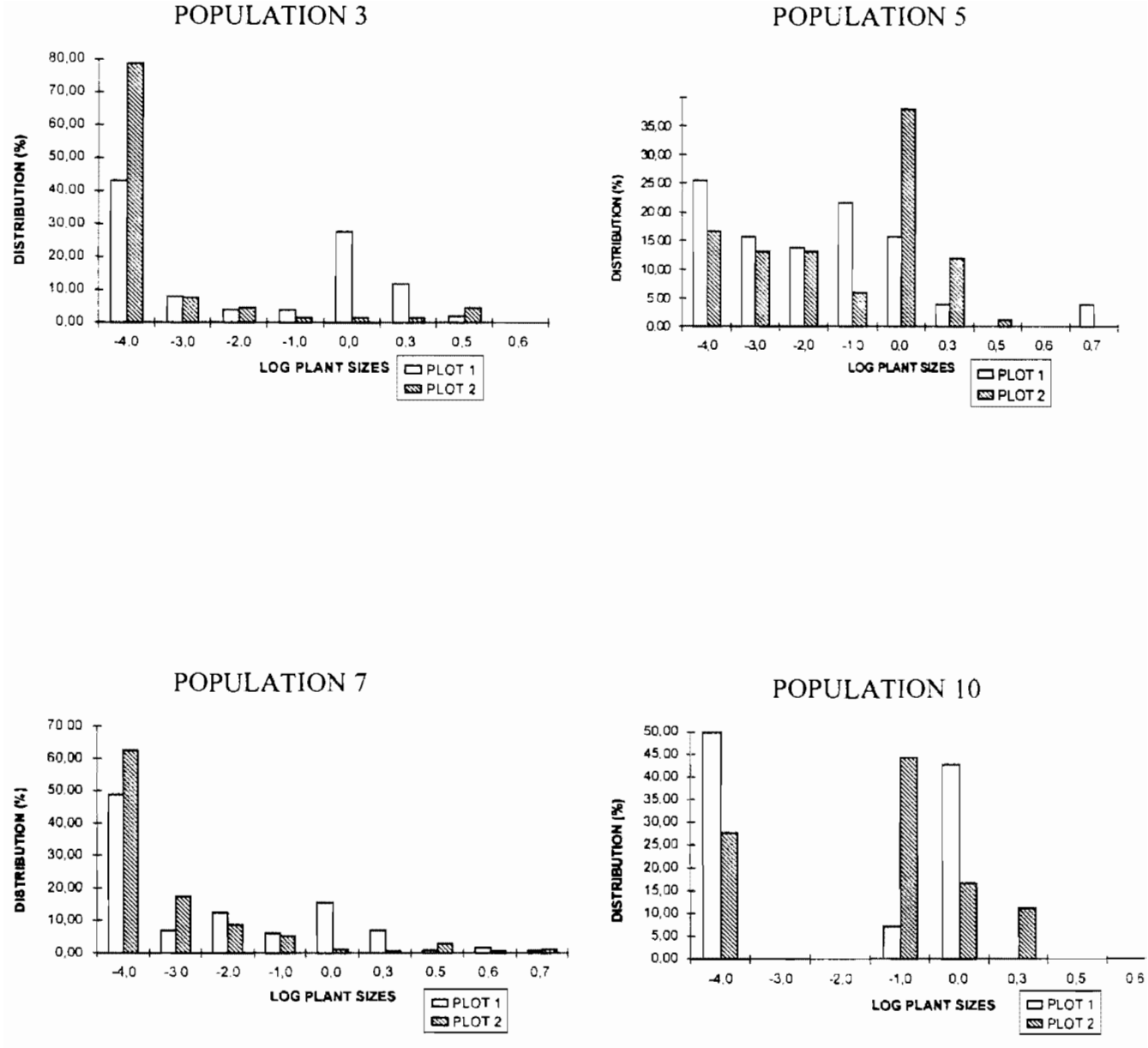

Figure 4.2 Size class distributions of the four medium sized populations sampled (populations 3, 5, 7 and 10). Two replicate plots were sampled in each population.

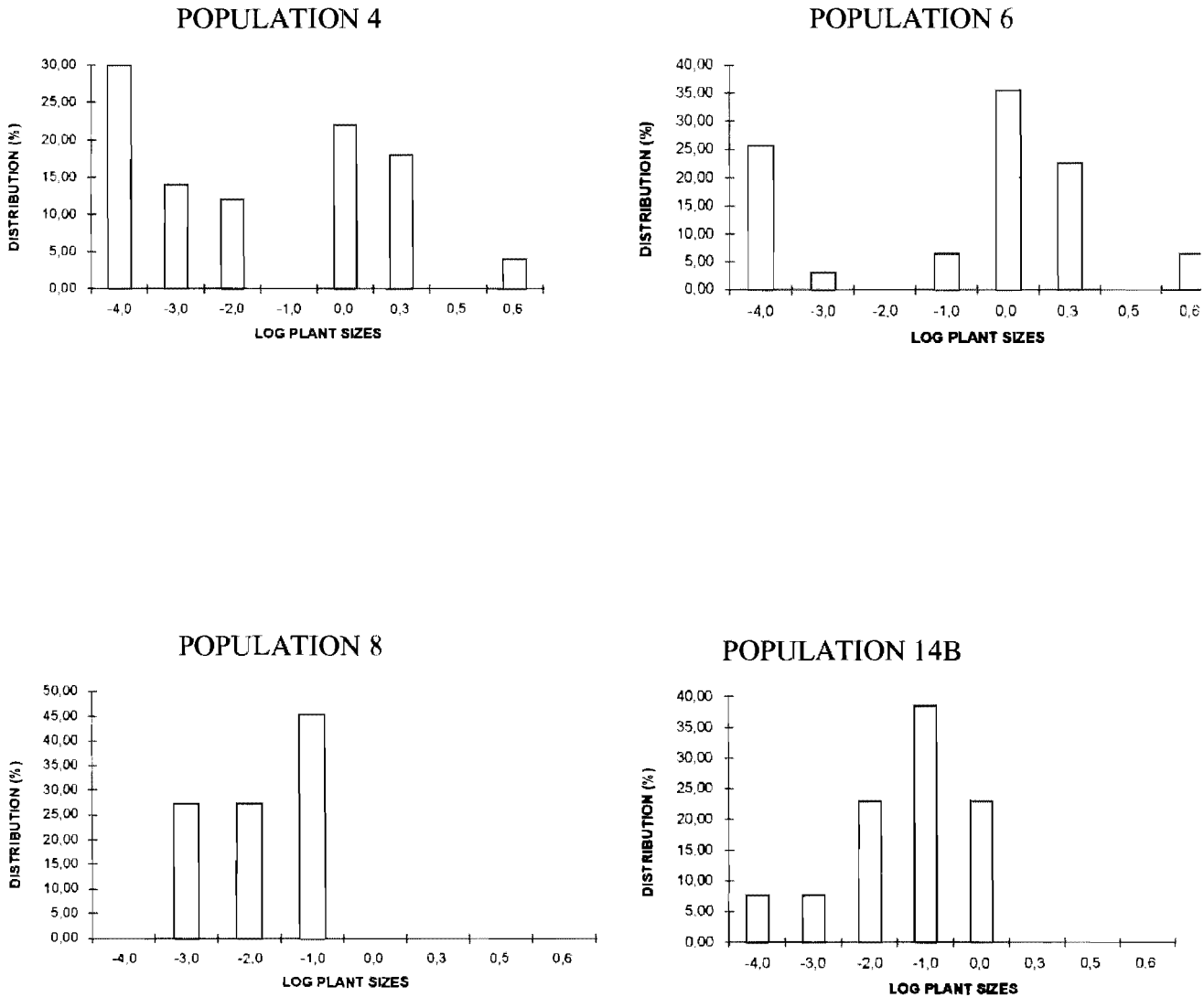

Figure 4.3. Size class distributions of the four small populations sampled (populations 4, 5, 8 and 14). One plot was sampled in each population.

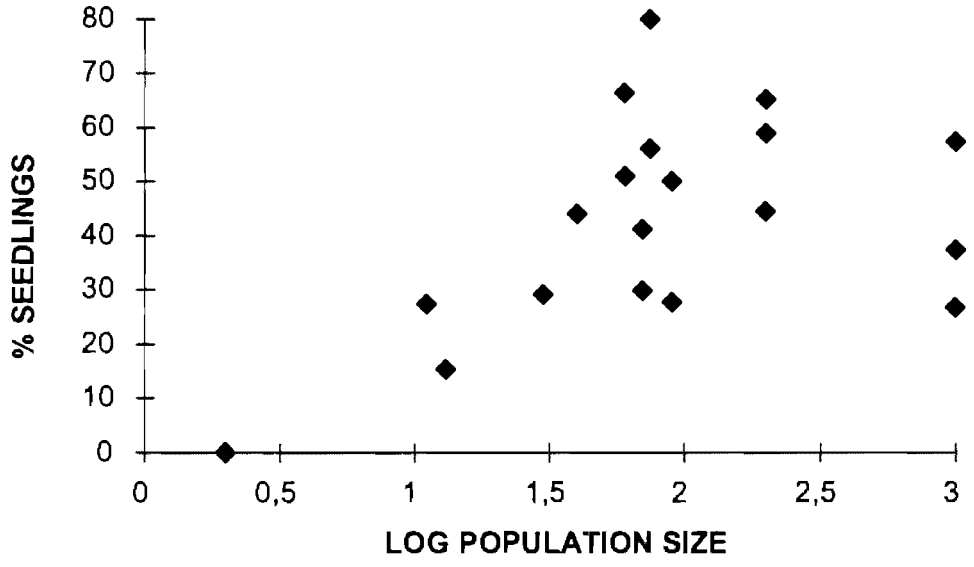

Figure 4.4. The percentage seedlings of the different sized populations plotted against the log of the population size for each replicate within each population ($p<0.05$, $r = 0.47$).

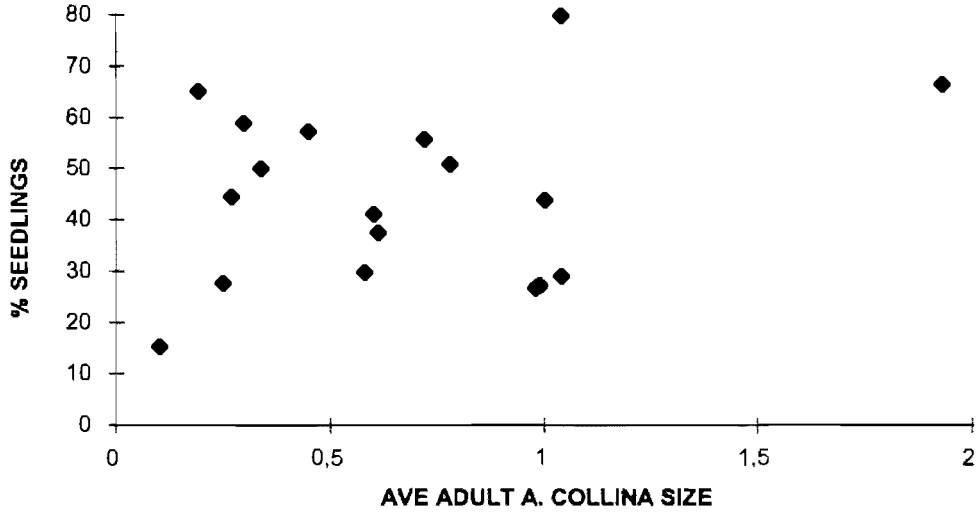

Figure 4.5. The average adult *A. collina* size (m^2) plotted against the % seedlings in each plot. Regression analysis shows no relationship between them.

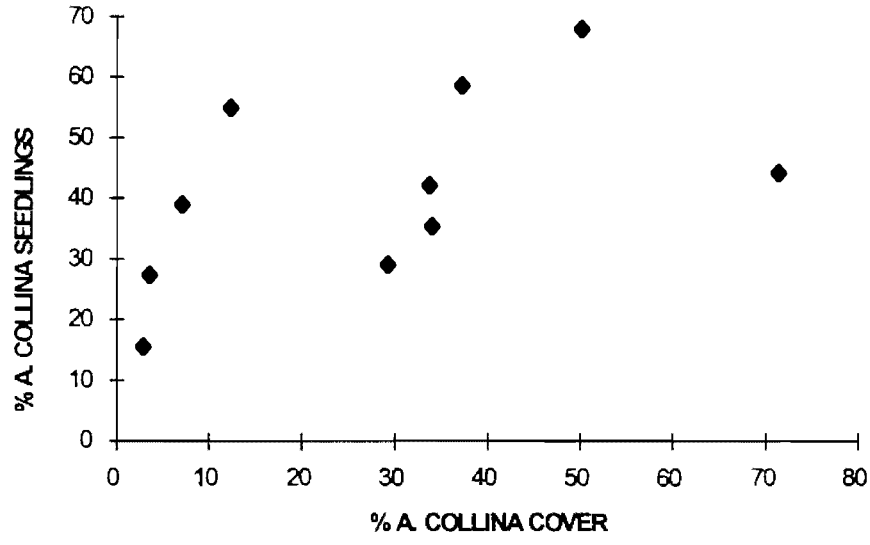

Figure 4.6. % *A. collina* cover plotted against the % seedlings in each plot. Regression analysis shows no relationship between them

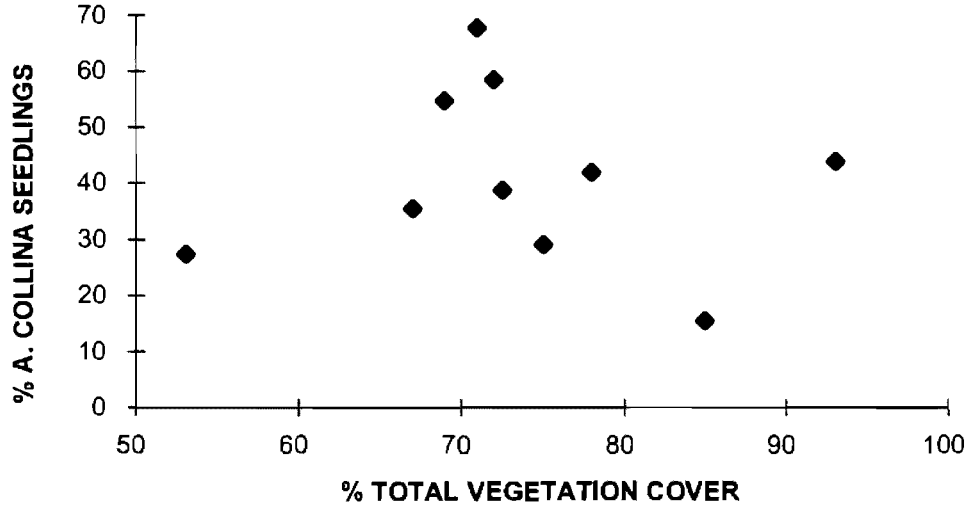

Figure 4.7. % total vegetation cover plotted against the % seedlings in each plot. Regression analysis shows no relationship between them

4. DISCUSSION

Of the number of seeds germinating under natural conditions only a small proportion of seedlings can be expected to survive until they are established. This is as a result of their susceptibility to environmental conditions during the seedling stage. The drop off measured from the percentage seedlings to the percentage of intermediate sized plants in populations of *A. collina*, therefore, follows this pattern.

The fact that there is a quantity of seedlings present and that there is a range of different size classes in each population indicates that intermittant germination of *A. collina* seeds takes place continuously.

Since *A. collina* is myrmecochorous its seeds are buried by ants and remain dormant until such time as the correct cue causes germination. Myrmecochorous seeds are buried by ants as a mechanism to avoid predators and fire and many will germinate after fire. Although no work has been done to indicate whether the germination cue for seeds of *A. collina* is fire it has been shown in many cases that soil stored seed banks of many fynbos species, including members of the Proteaceae, Restionaceae, Bruniaceae, Fabaceae and Rutaceae (Blommaert 1972, Jeffery et al. 1988, Bond et al. 1990, De Lange and Boucher 1990, Musil and De Wit 1990, Musil 1991) are stimulated to germinate either directly or indirectly by fire for a number of reasons. These reasons include increased temperatures during fire, increased fluctuations in diurnal soil temperatures and the effects of chemicals found in smoke. The fact that there is a peak in the size class distribution between $0.1m^2$ and $1m^2$ of canopy area in most plots sampled indicates that a higher number of seedlings survived to become adults at some point in the past. A logical explanation for this would, therefore, be that the last fire in the area would have stimulated germination of the seed bank in the soil which would have resulted in a much higher seed germination than normal resulting in a larger number of seedlings surviving to adulthood.

A fire moving through an area will leave patches of unburnt vegetation. The plots which did not show this peak of adult plants were probably those that escaped the fire and therefore did not have an increase in seed germination at this point.

Looking at the results in more detail however it was found that the percentage of *A. collina* seedlings in each plot was related to the overall population size. The proportion of seedlings increased from a minimum in small populations to a maximum in medium to large populations declining slightly in the largest population. The decline in the largest population may have been due to harsher environmental conditions since this population was situated in a dune slack which would have been protected from the cool winds from the sea in summer resulting in a hotter microclimate than in other parts of the study area.

Since there was a positive relationship between population size and the percentage of *A. collina* plants which were seedlings which could not be related to the average *A. collina* size, the density of *A. collina* cover and the total vegetation cover (Figures 4.5, 4.6 and 4.7), other explanations had to be found.

Menges (1991) found a similar result in a study of *Silene regia*. He suggested two possible explanations for this pattern:

1. Genetic effects - eg. inbreeding depression,
2. Demographic effects - eg fewer pollinators in smaller populations than larger.

The results of this study support the prediction of reduced viability of small populations since seedling recruitment was proportionally lower in small than in large populations of *A. collina*. This suggests that a minimum population size may indeed exist for this species. In the following chapters genetic and demographic factors possibly contributing to population size effects were investigated.

CHAPTER 5

DEMOGRAPHY OF A. COLLINA:

1. INTRODUCTION

Genetic problems in the conservation of small populations have been heavily emphasized in the conservation literature. Demographic consequences of small populations should, however, not be underestimated nor overshadowed by genetic studies since demographic factors may be of more immediate importance than genetic factors in the survival of a species (Lande 1988). For example seed predators in over abundance can significantly reduce seedling recruitment in a population (Andersen and Ashton 1988) while population density can affect pollinator and predator attractiveness of the population as a whole (Augspurger 1981, Menges 1990).

In the previous chapter (Chapter 4) it was found that seedling recruitment increased with population size. From a demographic point of view there are a number of factors which could explain this relationship. Seed set may be poor in small populations because of reduced pollinator visits. Alternatively seed escape from predators is more likely in large populations where predators are satiated. Pollination success and predation rates will affect seed production which could also be affected by competition between individuals of *A. collina*.

It was therefore necessary to study these aspects of reproduction in *A. collina* to attempt to find a demographic explanation for the different levels of seedling recruitment in different sized populations.

In this chapter I study the effect of population size on flowering phenology, capsule set and pre-dispersal predation and look specifically at the following:

- seasonal variation in flower numbers and inflorescence predation
- variation in duration of flowering in relation to plant and population sizes
- variation in inflorescence predation, in relation to the number of individuals in a population and season,
- variation in seed production dependent on population size and
- effects of competition between *A. collina* individuals.

The aim of this chapter is to attempt to identify those population size effects which can be explained by demographic factors.

2. METHODS

2.1. Peak Flowering And Length Of Flowering

The aim of this study was to determine if there was a difference in the length of flowering time between different sized populations which may affect the number of successful pollinations. For this assessment each marked plant in eleven of the populations studied (pops 1, 2, 12, 10, 5, 7, 3, 8, 6, 4, 14) was visited at least once a month, when very few inflorescences were present, and once every two weeks in the build up to peak flowering time, through peak flowering time and during the immediate tailing off period. This study began in mid August 1988 and ended at the end of March 1989. On each visit the number of inflorescences on each marked plant was estimated to be in one of a series of ranges:

1-10, 11-25, 26-50, 51-75, 75-100, 101-200, >200.

It was necessary to use these ranges since the large number of plants visited on each occasion made it impractical to count all the inflorescences on each plant.

Once an individual had more than 50 inflorescences with open flowers it was regarded as becoming attractive to insects since at this stage the white inflorescences became conspicuous on the plant (albeit to the human observer). At the stage where there were greater than 200 inflorescences with open flowers on an individual the plant was highly conspicuous and although the number of inflorescences on large plants frequently exceeded a thousand, peak flowering time was defined as the period over which individuals had greater than 200 inflorescences with open flowers.

An assessment was made with respect to:

- Plant size vs duration of peak flowering time.
- Population size vs mean duration of peak flowering time of individuals
- Population size vs length of time 80% of individuals in a population had >200 inflorescences (i.e. length of peak flowering time).

The length of flowering was obtained by graphing the data collected in the field and then extrapolating the length of flowering from the x axis (Figure 5.1).

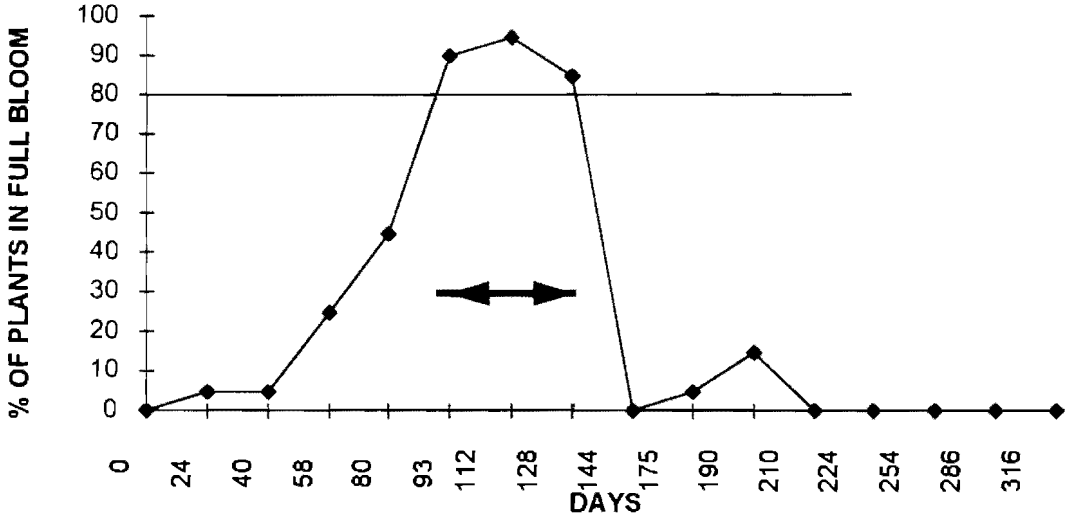

Figure 5.1. The percentage of plants in full bloom (i.e. >200 flowers) in one of the large populations sampled. The aim of this graph is to show how the curves are extrapolated to the X axis to determine the length of time each population has >80% of its individuals in full bloom. (In this example the arrows indicate the flowering time.)

2.2. Inflorescence Predation

In order to study the possible effects of pre-dispersal predation on seedling recruitment, the number of inflorescences which were damaged by predation was monitored. To determine seasonal changes in predation in populations of *A. collina*, the inflorescences collected to study seasonal changes in floral sex ratios from populations 5 and 7 were examined for signs of predation as described in Chapter 3. In November 1989, during peak flowering season, five inflorescences were collected from each of four individuals in each of the 11 marked different sized populations (only two individuals occurred in pop. 14 and therefore only 10 inflorescences were collected in this population). The percentage of inflorescences that had been damaged by a predator were calculated for each population. Damaged inflorescences were identified as those in which an insect larvae was found or in which the previous presence of a predator was detected by signs of grazing on the corolla tube or elsewhere in the inflorescence. The aim of this test was to estimate the effects of population size (if any) on predation. Populations were placed in three size categories (small, medium and large) and

the effect of size on inflorescence predation was analyzed by analysis of variance (ANOVA) using arcsin transformed data.

2.3. Seed Set Per Capsule

The number of seeds set could have a direct bearing on seedling recruitment. In order to collect seed capsules from *A. collina*, small branches were randomly broken off each marked plant in each population. Seed capsules of this species are highly cryptic since they tend to be hidden amongst the leaves of the plant and because they are the same colour as the leaves. In order to find capsules on *A. collina* plants it was necessary to deliberately search for them. This made it possible to randomly select branches without bias towards those which had more or bigger capsules than others.

Branches with a canopy diameter of approximately 15cm were collected. Capsules on the branches were then dissected and the number of seeds in each capsule was counted. Each seed capsule of *A. collina* has the potential to produce three seeds. The number of seeds per capsule of plants from the different sized populations were then compared using ANOVA. This experiment was carried out in 1988 and in 1989.

2.4. Competition

It could be argued that any affects found in the above experiments are related to competition and not population size since competition with neighbouring conspecifics is likely to increase with population size. Competition between *A. collina* individuals had, therefore, to be studied as well. Competition can be studied by calculating the relationship between the distance between two members of a species and the sum of their volumes (Welden et al 1988). A significant positive correlation between the distances and sum of the volumes means that larger neighbours are on average farther apart and indicates competition between them (Welden et al 1988).

The effects of nearest neighbours on plants was measured in populations 12 and 2. The distance between each marked plant and its nearest neighbour, measured from the stem of the one plant to the stem of the other, was measured as well as the canopy volumes of the two plants. The log of the volumes and the distance between the two plants was then regressed and tested for significance.

3. RESULTS

3.1. Peak Flowering And Length Of Flowering Time

In populations 12 and 2 there was a positive relationship between plant size and the length of time at which a plant had >50 inflorescences ($r=0.73$, $p<0.01$) (Figure 5.2).

During the survey however it was found that many of the small plants did not produce more than 50 inflorescences at any stage due to their small size. It was therefore decided that for further analyses plants categorized as small would be excluded.

Using only medium and large plants in each population the results show that there was no significant difference in the length of time over which plants of different sizes had >50 flowers (Figure 5.3).

There was also no difference between the length of peak flowering (i.e. 80% of individuals with >200 inflorescences) of the different sized populations (Figure 5.4).

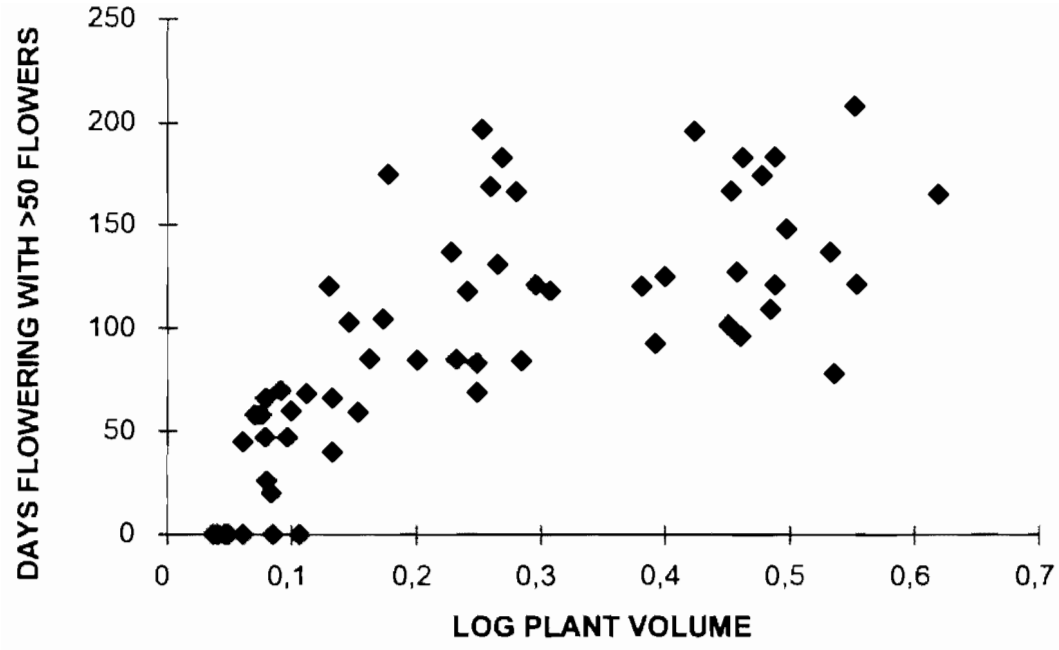

Figure 5.2. Plant volume regressed against days flowering. There is a positive relationship between the length of time an individual has >50 flowers and its size ($p<0.01$, $r = 0.73$).

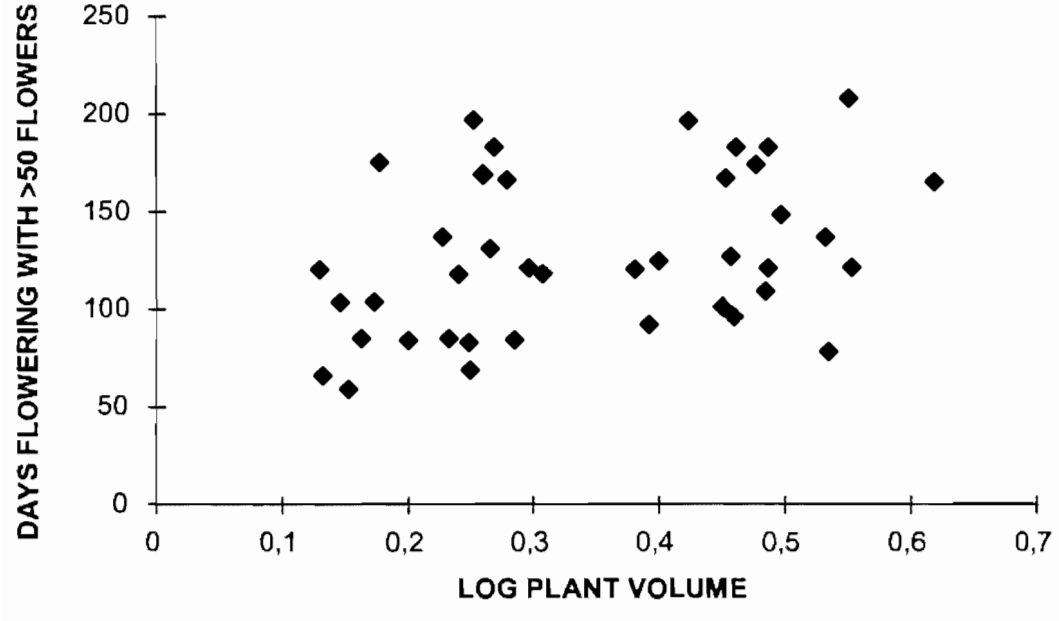

Figure 5.3. Plant volume regressed against days flowering using medium and large plants only. No significant relationship occurs.

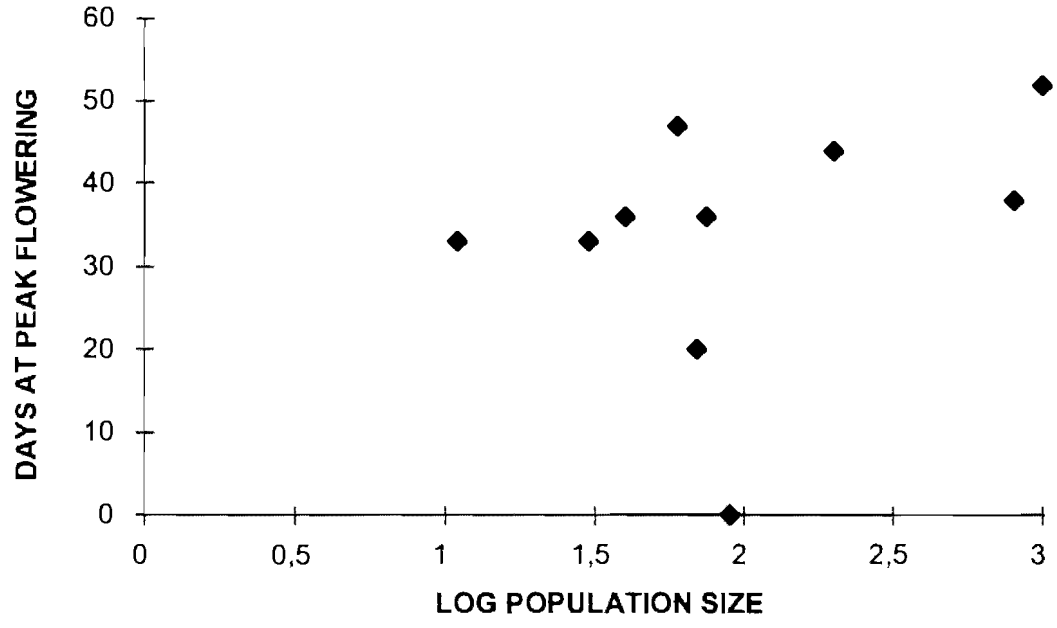

Figure 5.4. Days of peak flowering plotted against population size. There is no significant difference between the length of peak flowering of the different sized populations

3.2. Inflorescence Predation

There is a significant difference in percentage predation of inflorescences in the different sized populations (ANOVA, $P<0,01$) (Figure 5.5 and Table 5.1). Figure 5.5 shows that once a population reaches a size of approximately 30 individuals the percentage of inflorescences that are eaten increases. Once a population reaches 90 to 100 individuals the predation rate is once again reduced.

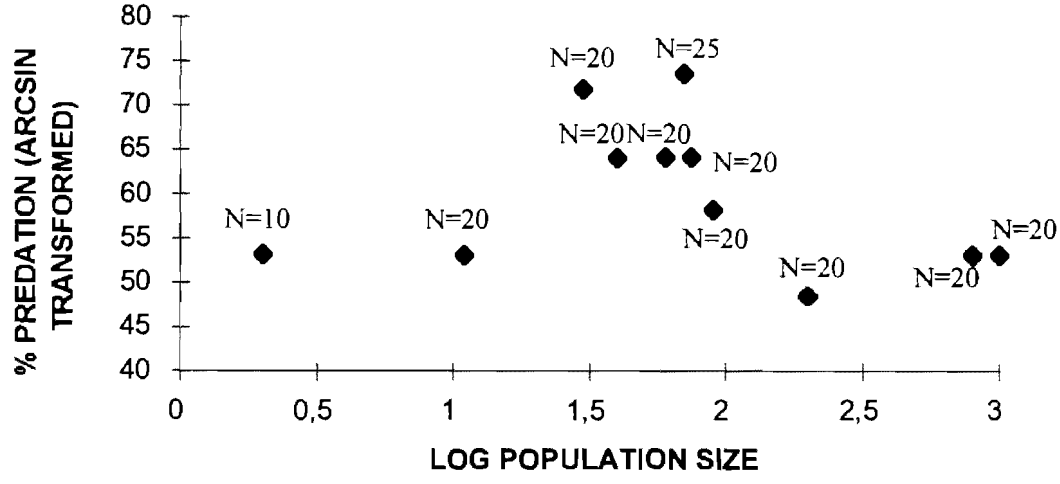

Figure 5.5. The percentage of inflorescences eaten (arcsin transformed) in the different sized populations monitored.

Table 5.1. One way ANOVA of the percentage predation of inflorescences in the different sized populations (arcsin transformed)

Sources	SS	d.f.	F-Ratio	Sig. Level
Between Pops	568,43	2	16,64	0,0014
Within Pops	136,64	8		
Total	705,07	10		

Percentage predation in populations 5 and 7 increased from June and July (16.3% and 2.8% respectively) to a peak in October and November (95.6% and 85% respectively) (Figure 5.6). This coincided with the peak flowering season of *A. collina*.

3.3. Seed Set Per Capsule

Plant size did not significantly influence seed set per capsule (obtained from data from populations 12 and 2) (Figure 5.7).

When the populations were grouped into different size classes (viz. small, medium and large) there were significant differences in the number of seeds set per capsule in 1988 (ANOVA) (Table 5.2).

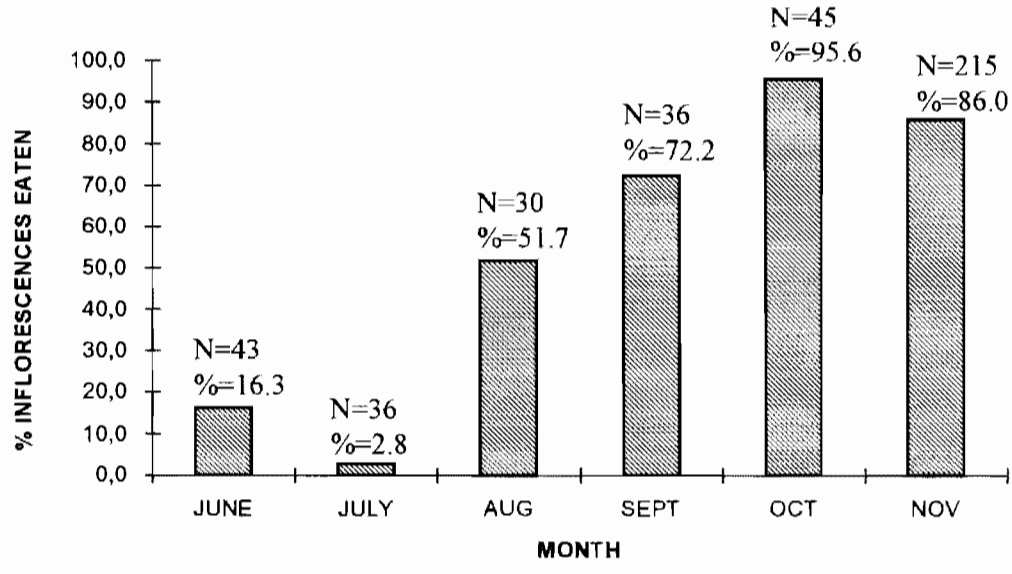

Figure 5.6. Seasonal changes in the percentage of inflorescences eaten presented as the percentage of inflorescences damaged by predation each month.

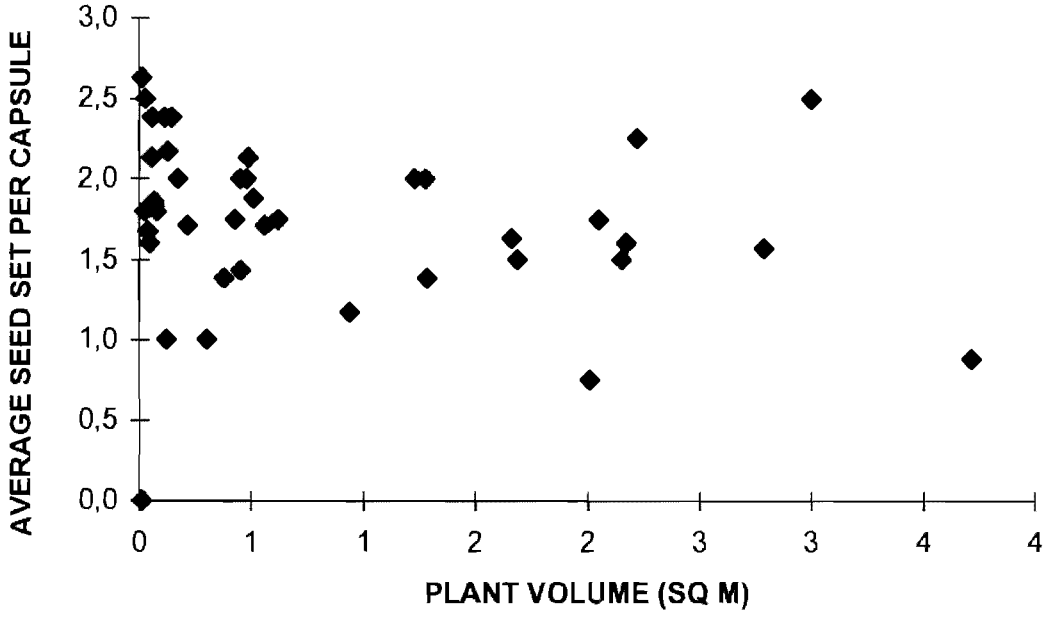

Figure 5.7. Plant size regressed against seed set per capsule. No significant influence was found.

Table 5.2. One way ANOVA of the number of seeds set per capsule in the different population size classes (small, medium and large) for the 1988 data

Sources	SS	d.f.	F-Ratio	Sig. Level
Between Size Classes	16.58	2	13.51	0,000
Within Size Classes	662.88	1080		
Total	679.46	1082		

Figure 5.8 shows the percentage of capsules in each population size class producing 3,2,1 or 0 seeds for the 1988 data. The figure shows a swing from a higher percentage of capsules producing 2 seeds in the large populations to a higher percentage of capsules producing 1 seed in the medium and small populations. A similar graph depicting the 1989 data (Figure 5.9) however shows that the percentage of capsules producing 3,2,1 and 0 seeds is almost identical in the different population size classes.

THE EFFECTS OF POPULATION SIZE ON *AGATHOSMA COLLINA* (RUTACEAE) AND ITS CONSERVATION IMPLICATIONS

BY

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Master of Science

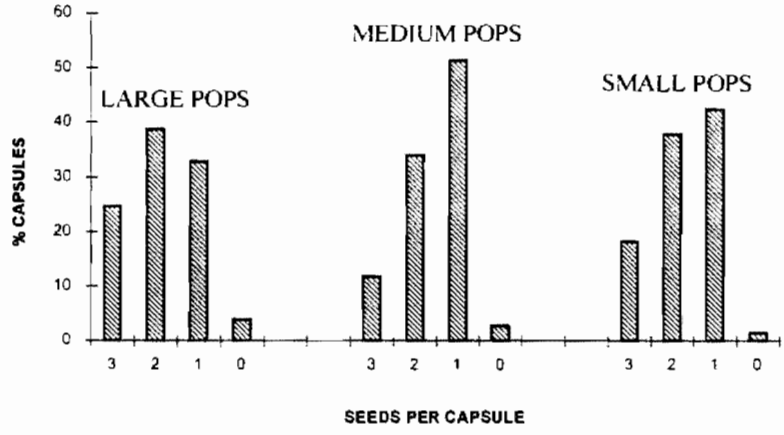

Figure 5.8. The percentage of capsules sampled in each population size class producing 3, 2, 1 or 0 seeds in **1988**.

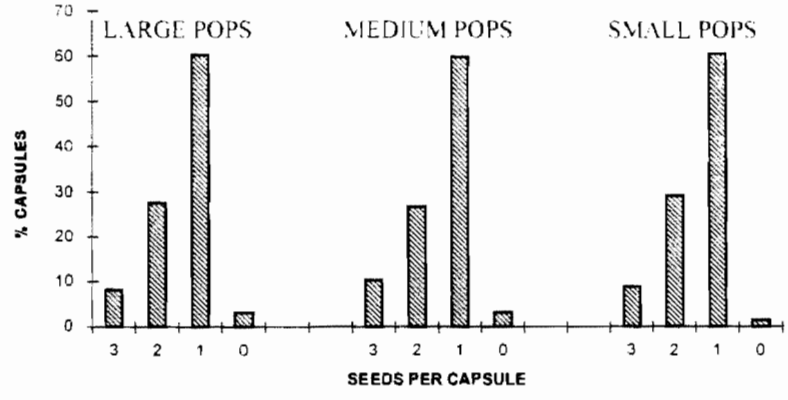

Figure 5.9. The percentage of capsules sampled in each population size class producing 3, 2, 1 or 0 seeds in **1989**.

3.4. Competition

When comparing the log of the sum of the volumes of neighbouring plants and the distance between them the results show that intraspecific competition does occur in *A. collina* ($r=0.609$, $p<0.01$) (Figure 5.10).

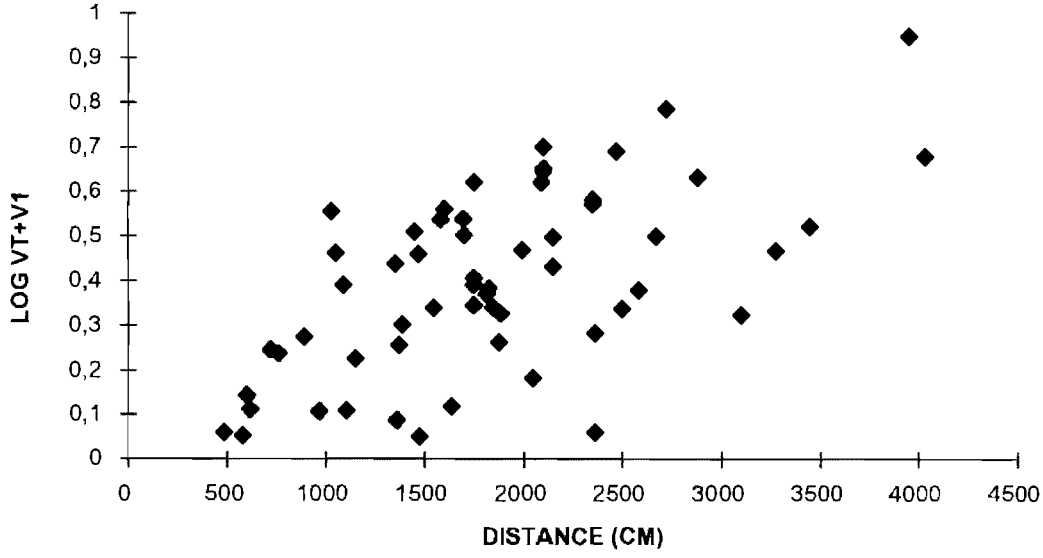

Figure 5.10. The log of the sum of the volumes of neighbouring *A. collina* plants plotted against the distance between them. There is a positive correlation ($P<0.01$, $r=0.609$).

4. DISCUSSION

4.1. Peak Flowering And Length Of Flowering

The length of flowering time and the length of peak flowering time between the different sized populations was not significantly different. This indicates that the attractiveness of different sized populations of *A. collina* to pollinators, in terms of length of flowering only, does not differ.

4.2. Inflorescence Predation

Differences in the levels of inflorescence predation were found between the different sized populations. Figure 5.5 shows that small and large populations had a lower predation rate than intermediate sized populations.

An hypothesis tested by Augspurger (1981) was that greater synchrony of fruiting within a population would reduce damage to the fruits and seeds of an individual by seed predators. This would occur because synchronized fruiting in the population would lead to satiation of the seed predators before a high percentage of the fruits in every individual had been damaged by predation (Janzen 1971). In addition Augspurger (1981) found that synchronously flowering populations of *Hybanthus prunifolius* had a significantly lower percentage of predation than an induced asynchronously flowering population.

A synchronously flowering, large population of *A. collina* produces a massive floral display. This display would serve to attract predators (and pollinators) and therefore a large number of inflorescences would be predated upon. Predator satiation, however, would reduce the percentage predation in such a population. Small populations, on the other hand, also have a large floral display but would not be able to rely on predator satiation by a large number of plants to reduce the predation percentage.

A synchronously mass flowering, large population of *A. collina* would, therefore, have increased seed survival due to predator satiation, when compared to a similar smaller population. In addition it could be expected that individuals flowering out of peak season would have a higher percentage predation of inflorescences when compared to the peak flowering season when a mass flowering display occurs.

It was found, however, that out of flowering season when fewest flowers were present on the *A. collina* plants damage by predators was at its lowest. The explanation for this would seem to be that season of predator activity and peak flowering time in *A. collina* may coincide. Out of flowering season, in winter, predator activity may be low thus reducing predation on the inflorescences at this time. A seasonal decline in insect predators could be due either to effects of cold weather on insect activity or food availability.

The lower levels of predation found in the smallest populations fits the prediction that scarcity reduces the risk of discovery (Janzen 1971).

The predator when searching for a host plant is more likely to find a large population than a small one. Small populations (i.e. less than approximately 30 individuals according to the results obtained: Figure 5.5) appear to be less likely to be discovered by predators and it can therefore be expected that the predation rate would be lower than in the larger populations. Medium sized populations are more likely to be discovered by predators than smaller populations but they may not be large enough to cause satiation of predators and thus reduce the percentage of inflorescences which are eaten.

The results here are consistent with Janzens arguments, they do not, however, show a correlation with increased seedling recruitment and increasing population size as found in chapter 4.

4.3. Seed Set Per Capsule

The 1988 and 1989 results differ markedly. Figure 5.8 representing the 1988 data indicates that the large populations studied produced a higher proportion of capsules with 3 and 2 seeds than the medium and small populations. Such a result would be expected for two reasons. From a demographic point of view large populations produce a larger flowering display and would therefore attract greater numbers of pollinators thus increasing the number of successful pollinations (Augspurger 1981). From a theoretical genetic point of view large populations are expected to have higher genetic diversity than smaller ones. This genetic diversity should result in more viable cross pollinations between individuals leading to a higher seed set. A higher proportion of non-optimal crosses would be expected in smaller populations which have reduced genetic variability.

The 1989 data show no differences in seed set per capsule. In fact figure 5.9 shows that the number of capsules producing 3,2,1 or 0 seeds in the different population size classes expressed as a percentage of the total capsules sampled are almost identical.

Climatic aspects may play a role in the difference between years by affecting

- the presence of pollinators in the region and
- environmental stress on the individual plants.

4.4. Competition

Competition may affect factors such as the length of flowering and seed production in the competing individuals. It was therefore necessary to examine competition between *A. collina* individuals.

Although competition does occur between *A. collina* individuals this is unlikely to affect the results obtained during the study. This is because three size classes of plants were studied, viz.: small ($<0,12m^3$), medium ($0,12$-$0,75m^3$) and large ($>0,75m^3$), and plants were chosen randomly for all experiments. Since the detection of competition is based on a comparison of the sizes of two neighbouring plants and the distance between them, by randomly sampling individuals of three different size classes and looking for trends over all the size classes, the effects of competition between sampled plants on the results obtained are minimized.

5. CONCLUSIONS

This chapter set out to look at the results of chapter 4 (viz.: increasing population size was found to correlate with seedling recruitment) from a demographic point of view in an attempt to explain these results.

Length of flowering did not vary between different sized populations so there was no population size effect on duration of pollinator attractiveness. The pattern of pre-dispersal predation between the populations, although consistent with predator satiation theory, did not correlate with the seedling recruitment results.

The effect of population size on seed production was not pronounced although some demographic effects of population size were detected during the study. A trend of reduced seed set in smaller populations was found but this was only significant in one of the two years in which the study was carried out.

Thus no single aspect of the demography of *A. collina* indicated a consistent population size effect each year. However, cumulative demographic effects may be important. Continual minor population size differences in seed set, for example, may result in major differences in the long term, while cumulative population size effects of a series of different demographic aspects may produce a significant long term effect.

It is important to note that this study was restricted to seed and seedling production and no studies of germination, seedling growth and survival were carried out. These are also areas where genetic or demographic consequences of small populations could be expressed but unfortunately due to the limited scope of this study not all these aspects could be addressed.

CHAPTER 6

GENE FLOW AND POPULATION STRUCTURE

1. INTRODUCTION

As indicated in chapter 1, the genetic neighbourhood size will determine the amount of substructure in a population. This will give an indication of the amount of gene exchange within and between populations. If neighbourhood sizes are small, inbreeding and outbreeding effects may be more important than where neighbourhood sizes are large since large neighbourhood sizes would have less of a restrictive effect on gene flow distances than small ones. The aim of this chapter, therefore, is to attempt to estimate the genetic neighbourhood size of *A. collina* (i.e. when can a population be considered fragmented?)

The main components of the neighbourhood size equation of Wright (1946) are gene flow and population density which therefore warrant further study here.

2. GENE FLOW

Levin and Kerster (1974) distinguish between potential gene flow and actual gene flow. The deposition of pollen on a stigma or the dispersal of a seed by ants is referred to as potential gene flow. Actual gene flow is the fertilization of the ovule by deposited pollen or the germination of seed resulting in a reproducing adult. Pollen viability, for example, if it was low, would affect measurement of actual gene flow but would not necessarily be included in estimates of potential gene flow. Although electrophoretic methods have been used to follow marker genes in order to study gene flow in plants (e.g. Schaal 1980), electrophoretic techniques tried on *A. collina* did not identify genetic markers (Chapter 8).

In the absence of appropriate genetic markers, direct observation of the movement of pollen and seed affords valuable insight into potential gene dispersal (Levin and Kerster 1974, Schaal and Levin 1978). Potential gene flow distances are, if anything, an exaggerated estimate of gene flow resulting in a larger neighbourhood size than would be expected through actual gene flow calculations. There may, however, still be chance long distance dispersal events which would be rare and not picked up by a study such as this.

The components of gene flow in plants are pollen and seed dispersal and in some cases vegetative dispersal of ramets (Golenberg 1987). This study concentrated on the dispersal of pollen and seed only because ramets of *A. collina* do not resprout or sucker in the populations studied (pers. obs.).

Pollen and seed dispersal are very often either exclusively local or highly leptokurtic (Levin and Kerster 1974) which would imply quite limited gene flow (Hamrick 1982) and may encourage inbreeding.

2.1. Pollen dispersal

Pollen dispersal in *A. collina* is affected by insects. Foraging insects tend, for reasons of energetics, to visit adjacent flowers to collect pollen (Lack and Kay 1987). An increase in plant density in a population may result in the pollinator foraging exclusively in one population (Levin and Kerster 1974) causing a lower outcrossing rate than in less dense populations (Hamrick 1982, Handel 1983). Plant density per unit area and the size of the population, therefore, should influence the incidence of inter-population pollinator flights.

2.2. Seed dispersal

Seeds of the Diosmeae are dispersed ballistically when the capsule dehisces (Pillans 1950). In addition, 95% of the Rutaceae, including *A. collina*, are myrmecochorous (Slingsby 1982). Myrmecochorous seed dispersal distances are very short (Bond and Slingsby 1983) resulting in restricted gene flow from this source.

Dispersal by ants is characterized by rapid seed discovery and removal (Bond and Slingsby 1983). This rapid removal of seeds and subsequent burial has been shown to reduce the incidence of predation and is essential for the survival of the seeds and therefore for the maintenance of effective gene flow in several species of Proteaceae (e.g. Bond and Slingsby 1983). In the case of the Rutaceae *A. betulina* and *A crenulata* are two species which have been studied by Blommaert (1972) and it was found that most dispersed seeds were eaten by mice and birds. Active myrmecochory may, therefore, reduce the predation of the ballistically dispersed seeds by rodents.

2.3. Neighbourhood Size

The isolation by distance model of Wright (1946) is based on the assumption that populations do not move in space. This is not necessarily the case but it is felt that the movement of *A. collina* populations is small enough to be ignored for the purposes of this study. Movement implies the nett movement of pollen or seed in a given direction (Levin and Kerster 1971). Pollinators were only observed on *A. collina* on windless or almost windless days. It is unlikely, therefore, that pollinator movement between plants will be in any particular direction and therefore the nett movement can be assumed to be 0.

Ballistic seed dispersal is random since the capsules are situated throughout the plant and dispersal away from the plant will be in all directions, nor is there any reason to expect bias in directional movement of seeds by ants.

According to Wright's (1946) equation for outcrossed hermaphrodite plants, neighbourhood size can be expressed in terms of area covered by the neighbourhood or in terms of the number of individuals in the neighbourhood (Richards 1986). The equation for the neighbourhood area (N_a) is expressed as follows:

$$N_a = 4\pi\sigma^2 \qquad \text{.....1}$$

where σ^2 = variance of pollen or seed dispersal distances

The neighbourhood size (N_e), i.e. number of individuals, is given by:

$$N_e = 4\pi\sigma^2 2d \qquad \text{.....2}$$

where d = the plant density within the population being studied.

Equation 2 has been used to calculate the neighbourhood sizes using ant dispersal, ballistic dispersal and pollinator flight distances separately (Beattie and Culver 1979) to produce 3 estimates of neighbourhood size.

Levin and Kerster (1971) combined the different dispersal mechanisms into one equation:

$N_e = 12.6d[(\sigma p^2 + \sigma s^2)/2]$ 3

where σp^2 and σs^2 describe the variances of pollen and seed dispersal distances respectively.

In the case of *A. collina* σs^2 consists of two components, viz. ballistic and myrmecochorous seed dispersal. Therefore a more effective equation for the calculation of neighbourhood size in this case would be:

$N_e = 12.6d(\sigma p^2 + \sigma b^2 + \sigma m^2)/3$.....4

or

$N_e = 4.2d(\sigma p^2 + \sigma b^2 + \sigma m^2)$.....5

where σb^2 and σm^2 describe the variances of seed dispersal resulting from ballistic dispersal and myrmecochory respectively.

But pollen dispersal only accounts for half the amount of genetic dispersal to that contained in seeds and this should be taken into account. Beattie and Culver (1979) worked on *Viola*, a plant similar to *A. collina* in that it is bee pollinated and its seed is ballistically and ant dispersed. In their calculations of neighbourhood size resulting from pollen dispersal only, Beattie and Culver (1979) halved the final result.

Levin and Kerster (1971) included half the pollen dispersal variance in their equation, therefore:

$N_e = 4.2d(\sigma p^2/2 + \sigma b^2 + \sigma m^2)$.....6

Neighbourhood area would be expressed as follows:

$N_a = 4.2(\sigma p^2/2 + \sigma b^2 + \sigma m^2)$.....7

3. METHODS

3.1. Pollen dispersal

Many experiments have been done which are concerned with the transfer of pollen or the flight distances of pollinators as a measure of potential gene flow in plant populations (Schaal 1978, Waser and Price 1982, Charlesworth and Charlesworth 1987, Schlissing and Turpin 1971, Handel 1976 and Colwell 1951). The most effective techniques described are those using fluorescent dyes (Waser and Price 1982) and those using I131 as pollen markers (Schlising and Turpin 1971, Turpin and Schlising 1971). The fluorescent dye method involves dusting the dehiscing anthers of the plant with dye and then searching for dye on the flowers of other plants in the vicinity. Waser and Price (1982) showed that the transfer of pollen and dye in *Ipomopsis aggregata* was very similar and an accurate measure of pollen flow. Here I attempted to measure pollen dispersal using fluorescent dye.

Two colours of dye were used, magenta and yellow. Each dye colour was deposited on all dehiscing anthers in a group of approximately five inflorescence on one individual at dusk on the first evening. Two individuals were therefore marked on each occasion. The experiments were conducted under light wind conditions since insects were inactive on windy days. The following evening after dark the site was visited and the dye was sought on inflorescences within the same plant and on nearby plants using a portable ultra violet lamp. The dye was left for more than 24 hours to ensure that any nocturnal pollination by moths or any other unexpected insects was recorded.

Pollen dispersal was also estimated using neighbour analyses following the assumption that insects visiting these plants would more often move to neighbouring individuals and dispersal would not have taken place over long distances as discussed at the beginning of this chapter. Accordingly, the distance of the nearest neighbour to 60 marked plants in populations 12 and 2 were measured and used as an estimate of potential pollen dispersal. This provides a conservative estimate of pollen flow.

3.2. Pollen viability

The pollen from one anther from each of ten flowers on ten different inflorescences from different plants was stained for viability on a glass microscope slide using Alexander's stain (Alexander 1969). Two hundred pollen grains on each slide were counted and the percentage of non viable grains in each sample of 200 was calculated.

3.3. Seed dispersal

Ballistic seed dispersal distances were measured by collecting small branches holding ripe seed capsules from the plant. When the seeds inside a capsule become ripe the testa changes colour becoming hard and black. Ripe capsules can be distinguished from others since the black testa of the seed shows through the capsule wall at this stage. The capsules were returned to the laboratory where they were allowed to eject their seeds. The bases of the branches were inserted into the necks of bottles at the average height at which they are naturally found on plants (0.75m from the ground). The bottles were placed in the centre of white sheets and left in a warm room. Seed dispersal distances were measured at intervals throughout the day and the seeds removed from the sheet to avoid confusion. The dispersal distance was the distance the seed was ejected from the base of the bottle.

Despite repeated attempts to attract ants to seeds, I observed very few seed movements by ants. In order to get an estimate of gene flow by myrmecochory, should it even be important in *A. collina* populations, I used estimates of seed dispersal from other fynbos studies.

3.4. Density

The densities of the marked populations used throughout the study were measured. Circular or semi-circular plots with a 10m diameter were used. In the large and medium sized populations two plots were sampled while in the small plots it was not possible to include more than one plot. The density of *A. collina* in each plot was then expressed as the number of reproducing *A. collina* plants per m^2. A reproducing individual was counted as being one with a canopy volume of $>0.01m^3$ since below this volume no plants were found that had ever flowered.

4. RESULTS

4.1. Pollen dispersal

Fluorescent dye movement was not observed at all on four different occasions using fluorescent dye and a UV lamp.

With no discernible pollen movement using this method the nearest neighbour distances were used instead. The average distance to the nearest neighbour in populations 12 and 2 was 0,56m (s^2 = 0,34, n = 60)

Pollen viability calculated from the Alexanders stain technique was high. The percentage of non-viable pollen in ten samples of 200 pollen grains averaged only 1,65 (Highest = 3,5%).

4.2. Seed dispersal

Ballistic seed dispersal of 45 seeds averaged 1.116m (s^2 = 0,24). Figure 6.1 shows the dispersal distribution of the seeds.

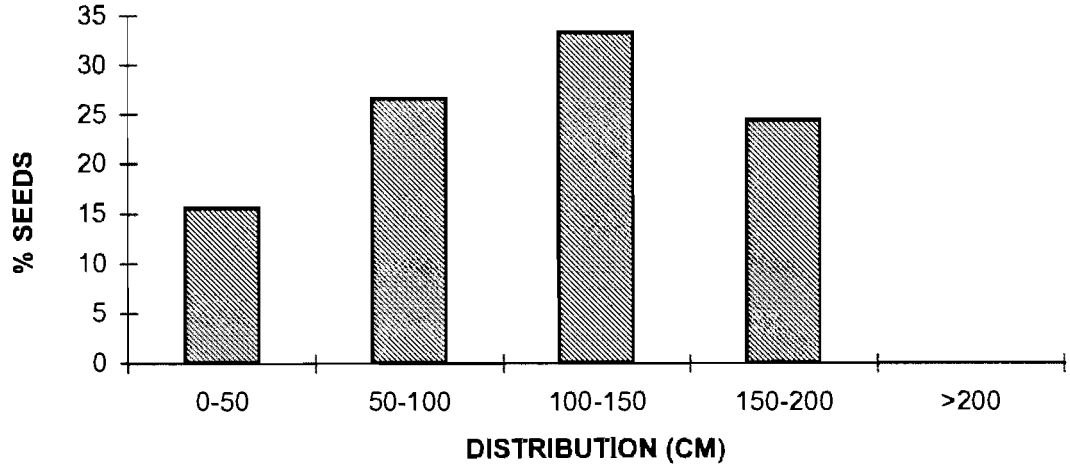

Figure 6.1. The dispersal distribution of ballistically dispersed seeds of *A. collina*. (n=45)

Since myrmecochorous dispersal of *A. collina* seeds were not observed, seed dispersal due to myrmecochory was estimated from work described in three references.

- Berg (1975) using direct observation in the field reported 6 distances for different species dispersed by different ants. The average distance that the seeds were dispersed was 3,52m ($s^2 = 2,31$).

- Slingsby and Bond (1985) using seedling distributions after fire estimated the mean dispersal distance of seeds of *Leucospermum conocarpodendron* as 2,52m ($s^2 = 5,15$).

- Andersen (1988) studying the dispersal of *Acacia suaveolens* seeds found the average dispersal to be 2,1m ($s^2 = 2,56$).

Since seed dispersal was not measured directly for *A. collina* the average of the three standard deviations mentioned will be used as an estimate of the standard deviation of the species being studied, i.e. $s^2 = 3,34$.

4.3. Density

The densities of the different populations measured are given in Table 6.1 with the average density being 0.45 plants/m^2 ($s^2 = 0.20$)

4.4. Neighbourhood area

Calculating neighbourhood area using the method of Beattie and Culver (1979) on *Viola* (equation 2) the following results were obtained:

Using pollen dispersal distances, $N_a = 4,23/2 = 2,12m^2$

Using seed dispersal distances:

- Ballistic dispersal, $N_a = 3,05m^2$
- Myrmecochory, $N_a = 41,97m^2$

Using the combined equation (7) the neighbourhood area would be:

$N_a = 15.75m^2$

or if myrmecochory were negligible using the equation

$N_a = 12.6(\sigma p^2/2 + \sigma b^2)/2$

$N_a = 5.166m^2$

4.5. Neighbourhood size

Neighbourhood sizes (equation 2) resulting from the different dispersal mechanisms for the different populations are given in Table 6.1.

When the combined neighbourhood size is calculated using equation 6 it can be seen that the large neighbourhood sizes resulting from myrmecochorous dispersal are reduced since the neighbourhood sizes resulting from the other mechanisms are small (Table 6.1).

Table 6.1. Neighbourhood sizes of the sampled populations of *A. collina*. The table includes neighbourhood size calculated from pollen, ballistic seed dispersal and myrmecochory separately and all of these combined as well as a combined neighbourhood size excluding myrmecochory.

POP NO.	AVE DENSITY Adults /m^2	NEIGHBOURHOOD SIZE (N_e) POLL	BALL	MYRM	COMBINED	NEIGHBOURHOOD SIZE (N_e) EXCLUDING MYRMECOCHORY
12	0,59	1,3	1,8	24,8	9,3	1,5
2	0,57	1,2	1,7	23,9	9,0	1,5
10	0,26	0,6	0,8	10,9	4,1	0,7
7	0,59	1,3	1,8	24,8	9,3	1,5
5	0,57	1,2	1,7	23,9	9,0	1,5
3	0,43	0,9	1,3	18,0	6,8	1,1
4	0,71	1,5	2,2	29,8	11,2	1,8
6	0,28	0,6	0,9	11,8	4,4	0,7
8	0,03	0,1	0,1	1,3	0,5	0,1
AVE	0,45	0,95	1,37	18,79	7,05	1,16
SD	0,20	0,43	0,62	8,59	3,23	0,53

If the combined neighbourhood size is calculated using the different densities found in the plots studied in each population it can be seen that neighbourhood size differs between sample plots within a population (Table 6.2).

Table 6.2 Neighbourhood sizes of the two different plots sampled in each of the medium and large populations of *A. collina* using the combined neighbourhood sizes of Table 6.1. This shows how different results are obtained within populations depending on density.

POP NO.		DENSITY Adults/sq m	NEIGHBOURHOOD SIZE (N_e)
12	PLOT 1	0,89	14,0
	PLOT 2	0,28	4,4
AVE		0,59	9,3
2	PLOT 1	0,76	12,0
	PLOT 2	0,38	6,0
AVE		0,57	9,0
10	PLOT 1	0,18	2,8
	PLOT 2	0,33	5,2
AVE		0,26	4,1
7	PLOT 1	0,73	11,5
	PLOT 2	0,45	7,1
AVE		0,59	9,3
5	PLOT 1	0,38	6,0
	PLOT 2	0,75	11,8
AVE		0,57	9,0
3	PLOT 1	0,63	9,9
	PLOT 2	0,23	3,6
AVE		0,43	6,8

5. DISCUSSION

Direct estimates of pollen movement proved difficult to obtain. *A. collina* has a massive flower display when it flowers (Figure 6.2). Augspurger (1980) found that mass flowering in *Hybanthus prunifolius*, yielded a reduced gene flow since the interplant movement was limited because pollinators remained on a single plant for long periods. She found that the major pollinator was attracted consistently only to a high density of floral resources. In her study Campbell (1985) found that 95% of moves by non specific pollinators were to nearest neighbours independently of species. Similarly in the case of *A. collina* the pollinators are likely to spend most of their time on one individual and if they move they may not necessarily move to another of the same species. Wasps tend not to be species specific (Steiner 1987) and since the pollinators of *A. collina* are a number of wasp and bee species (pers obs.) it is assumed that the pollinators of *A. collina* are probably not species specific. In the event of them moving to another plant of the same species it would be likely that they would move to a nearest neighbour which also had a mass flowering display.

Figure 6.2 An *A. collina* plant in full flower. Note the mass of flowers

Schaal (1974, 1975) in her studies of *Liatris cylindracea* found that dispersal distances of pollen by bees were small as bees tended to visit nearest neighbours when foraging resulting in population differentiation across very small distances. Schaal (1978) studying *Liatris pycnostachya* showed that at a given density the probability of two plants crossing was proportional to the distance between them.

Pollinators that forage indiscriminately can transfer pollen from one species to another, reducing the amount that reaches conspecific flowers.

It is possible that the reason for the lack of movement of the marker dye in the pollen dispersal experiment was due to weather constraints. Most insects were observed on hot calm days with little cloud cover. Such days are rare in the study area.

Assuming that a wind speed of $>25ms^{-1}$ and a cloud cover of >1 in a grade of 8 units as used by the Pretoria weather bureau is cause for a reduction in pollinator activity, then using data supplied by the weather bureau from the Agulhas weather station the number of optimal pollinating days can be calculated. The wind speed and cloud cover data collected at 14h00 was used since insect activity was best during the hot midday period. The optimal flowering time for *A. collina* (i.e. the time that most flowers are present on a bush) occurred in October and November. Using the data collected at the Agulhas weather station, it was calculated that there were only four days during this period in 1989 and none in 1988 in which optimal wind speed and cloud cover coincided. On the very few such days that did occur insect activity was markedly improved when compared to other days (pers. obs.). Pollen viability however was high so that pollen dispersal that did occur between plants would most likely have resulted in viable pollen being deposited on a stigma.

The rarity of such days and the difficulty in predicting them add considerably to the difficulties of studying pollination in fynbos. Johnson (1992) studying pollination in Cape petaloid monocots has had the same problem and has found evidence for pollinator limitation of seed set in many species.

Small quantities of insects were, however, present in the populations during most weather conditions so that at least some cross pollination must have occurred between plants at all

times, but with thousands of inflorescences per plant and approximately 25-30 flowers per inflorescence at optimum flowering time it is not surprising that little movement of the marker dye was detected. It was hoped however that at least some movement would be detected to give an indication of the sort of distances involved in pollen dispersal.

Neighbourhood area resulting from the three different mechanisms of dispersal varied considerably. The neighbourhood area resulting from estimates of pollen dispersal distances was only 2,12m^2 compared to ballistic dispersal of 3.05m^2, while that resulting from myrmecochorous seed dispersal was 41,97m^2.

Beattie and Culver (1979) found similar degrees of variation in *Viola* resulting from the different dispersal mechanisms. They however found that the largest neighbourhood area resulted from pollen dispersal (of six species of *Viola* the smallest neighbourhood area was 16.4m^2 and the largest 69.6m^2) and the smallest from myrmecochorous seed dispersal and concluded that pollination was more important than seed dispersal in determining neighbourhood area and effective population size. Field evidence during this study suggests that myrmecochory is rare and that most gene flow in *A. collina* was via ballistic seed dispersal. Ballistic seed dispersal may have been slightly overestimated since in the laboratory experiment there were no obstructions such as shrubs. The neighbourhood area observed by Beattie and Culver (1979) as a result of ballistic dispersal varied between 5.8m^2 and 8.8m^2 compared to 3.05m^2 in *A. collina*.

6. CONCLUSIONS

The important points which arise from this study are that:

- the different components of gene flow vary in their importance between species and that
- gene flow in *A. collina* is restricted to relatively short distances

6.1. The different components of gene flow vary in their importance between species.

Ballistic and myrmecochorous seed dispersal mechanisms are likely to be relatively poor systems for gene flow since they are restricted and are likely to result in substantial population differentiation. Neighbourhood area as a result of pollen dispersal, although small in *A. collina*, has the potential to be the largest. Beattie and Culver (1979) estimated neighbourhood area of *Viola* spp. resulting from pollinator flight distances to be up to 69.6m^2 while Young (1986) estimated neighbourhood areas for beetle pollinated *Diffenbachia longispatha* of up to 177713m^2. Rare long distance dispersal of *A. collina* pollen may increase neighbourhood area in this species.

Richards (1986) mentions two problems regarding neighbourhood size estimates.

1. Gene flow varies considerably over time depending on environmental factors.
   As has been shown the conditions which are best suited to *A. collina* pollination are windless, cloudless days. This combination is not frequent in the regions in which the habitat of this species is found. In fact in 1988 no such conditions occurred over the flowering period. Environmental conditions vary daily creating problems for neighbourhood size calculations. Authors tend not to mention environmental conditions when calculating neighbourhood size (Beattie and Culver 1979, Crawford 1984) since they are clearly not easily incorporated into any general equation. Comparisons of neighbourhood size between species and even within the same species on different days would therefore be difficult.

2. The second problem is that estimates depend heavily on density.

   With an increase in density there is an increase in neighbourhood size. This will vary between populations and even within large populations (Table 6.2). Neighbourhood size estimates are, therefore, very specific to a population and care should be taken when extrapolating the results to obtain estimates for different populations. Neighbourhood area is likely to be more constant since, although environmental conditions may vary, the dispersal mechanisms are likely to be similar. Having said this, though, it is clear that density plays a large role in the distance that pollen is dispersed (Lack and Kay 1987, Levin and Kerster 1974, Hamrick 1982, Handel 1983, Augspurger 1980, Schaal 1978).

6.2. Gene flow is restricted to relatively short distances.

The indication gained from this study is that gene flow in *A. collina* is restricted to relatively short distances only. It must be remembered, however, that neighbourhood areas may differ depending on weather conditions and population density and since *A. collina* occurs in a relatively stressful habitat small neighbourhood sizes are not unexpected.

This chapter indicates that gene flow in *A. collina* may be too short for substantial interchange among populations of this species. With very short dispersal distances as obtained in this study, if the entire area was regarded as a metapopulation, the sub-populations studied may well have been genetically isolated.

CHAPTER 7

INBREEDING AND FITNESS

1. INTRODUCTION
2. METHODS
3. RESULTS
4. DISCUSSION
5. CONCLUSIONS

1. INTRODUCTION

"An interesting empirical question in population biology is the extent to which the identity of an individual's mates affects its fecundity and ultimately its relative fitness" (Redmond et al 1989)

In any population of a species where pollen is transported from one individual to another, the distance of pollen transport will vary depending on many factors, e.g. population density (Levin & Kerster 1969, Schmitt 1983) and type of pollinators etc. The relatedness between the donor individuals and the recipient will also vary. This variation in relatedness may give rise to different reproductive success.

Inbreeding between genetically similar parent plants may expose deleterious alleles which would reduce the reproductive success of such a cross (Price & Waser 1979, Levin 1989). Levin (1984), for example, found that in *Phlox drummondii*, a spring flowering annual and normally self-incompatible species, seed viability was higher with cross- than self-fertilisation.

With short distance pollen and seed dispersal, the genetic relatedness of plants tends to decrease as the distance between them increases (Schaal 1974, Levin 1984, Levin 1989, Waser & Price 1989), therefore, if a particular species being studied is self-incompatible, close relatives may also be incompatible. If the genetic relatedness of plants decreases with distance then cross-compatibility between individuals will be positively correlated with distance (Levin 1989) as the effects of incompatibility decrease.

The size of a sub-population (or neighbourhood) within a metapopulation must play a role in determining the severity of the inbreeding effects found to occur within a metapopulation.

If the neighbourhood size is large then there would be a larger gene pool from which pollen could be obtained and the effect of inbreeding would be reduced when compared with a population with a small neighbourhood size. Plants genetically similar to each other, especially those of self-incompatible species, are expected to show a reduction in reproductive success and offspring survival when crossed (inbreeding depression) (Levin 1984, Levin 1989, Redmond et al 1989, Waser & Price 1989).

Within a small neighbourhood the gene pool would be reduced, the plants within the neighbourhood would be more closely related and therefore, the effects of inbreeding would be increased.

Outbreeding depression may occur when the individuals crossing are genetically too dissimilar for effective crossing to occur (Sobrevila 1988, Redmond et al 1989, Waser & Price 1989). This could occur as a result of isolation of two populations from each other resulting in different selective pressures on each population which could make them become genetically dissimilar over time. Selective pressures may also occur within a population increasing the genetic dissimilarity between individuals within the population.

Crosses between genetically too dissimilar individuals would be less effective than more closely related individuals.

Waser & Price (1989) found that in *Ipomopsis aggregata* if the pollen was transported from a plant too far away from the female then seed set was depressed. The optimal distance for pollen transport in terms of best seed set was very short, around 10m, indicating that outbreeding depression may result over very short distances.

In the above mentioned examples the authors have all correlated genetic distance between individuals of a species with the actual distance between them. The hypothesis which stems from this discussion therefore is that there is an optimum outcrossing distance between crossing individuals which would produce optimal reproductive output. This optimum outcrossing distance can be related to the genetic relatedness between the parent plants.

Sobrevila (1988) defines optimal outcrossing distance as that distance at which seed set reduction due to inbreeding and outcrossing is not present. It is unlikely however that in a plant population where inbreeding or outbreeding effects occur there will be an optimal distance at which these effects will not be present at all. A better definition of an optimal outcrossing distance is that if both inbreeding and outbreeding depression occur within a population, there should be an intermediate degree of in- and outbreeding at which their overall deleterious effects are minimised (Price and Waser 1979, Waser and Price 1983, Waser and Price 1989)

The aims of this experiment are:

- to discover the breeding system of *A. collina* with respect to possible self-incompatibility,
- to study fragmented populations to assess whether inbreeding is a problem and if so over what distance,
- to assess whether outbreeding is a problem and over what distances it occurs and
- to find out if there is an optimal outbreeding distance.

2. METHODS

In the spring of 1988 ten large maternal plants of *A. collina* (as defined in chapter 2, large plants had a canopy volume of > $0.75m^3$) within close proximity of each other were marked. At the same time groups of five large pollen donor individuals at various distances from the maternal plant were marked (viz.: nearest neighbour, 25m, 200m, 1km, and 7km) to be used as a pollen source.

On each maternal plant 1000 inflorescences, with buds with their first petals just beginning to unfurl, were covered with a nylon mesh bag to prevent natural pollination occurring. After 9 days when most of the stigmas were receptive (see chapter 2) the bags were removed and on each of the inflorescences all the stigmas that were present were pollinated. Pollen for these pollinations was obtained from the same maternal plant (i.e. self-pollination) and from the five donor plants at each of the different distances. Due to the large numbers of inflorescences needing to be pollinated the hand pollinations were carried out over a period of five days. Only individuals with receptive stigmas were pollinated. Those with still immature flowers were rebagged to be pollinated at a later stage.

Pollen for each cross pollination treatment was obtained from the five donor plants and on each of the 10 maternal plants two inflorescences were pollinated from each of the five donor plants. For each treatment, therefore, 10 inflorescences on each of the 10 maternal plants were pollinated (i.e. 100 inflorescences per treatment in most cases). For the nearest neighbour treatment however all the pollen for each of the ten maternal plants was collected from their one nearest neighbour. For the self pollinations the pollen was all obtained from the maternal plants themselves.

Of the 1000 inflorescences bagged a large proportion were damaged by predation. Predation of the inflorescences was in the form of insect larvae which were presumably deposited as eggs into the young buds prior to their being bagged. The larvae when hatched fed on the inflorescences often destroying all the flowers on an inflorescence. Any inflorescences showing signs of predation were excluded from this experiment and as a result it was not possible to pollinate 100 inflorescences for every treatment.

When the bagged inflorescences were collected from the plants at the end of February 1989 some had been damaged, were missing or had been broken off by the strong winds which occur in the region. The number of these were relatively low.

The sample size (N) for each treatment (Table 1) therefore represents the number of successful baggings which were carried out for each treatment taking these problems into account.

On each maternal plant 100 inflorescences were marked, but not bagged as a control to get an indication of natural pollination. Once these inflorescences had had sufficient time to be pollinated naturally, i.e. when their petals had died back and their stigmas were no longer receptive, they were bagged to provide similar conditions to the pollinated inflorescences, to protect the developing capsules and to catch them should they drop off.

On each maternal plant 100 inflorescences were left bagged without treatment to control for the possibility of agamospermy.

Pollination of the maternal plant was effected by collecting inflorescences presenting pollen from the donor plants. This was usually carried out after the morning ambient temperature had risen sufficiently to cause the anthers to dehisce (usually between 10:00am and 11:00am). This was to ensure the use of fresh pollen for the pollinations and not pollen which had dehisced the previous day.

The inflorescences were collected from the donor plants into plastic vials and transported to the maternal plant immediately. At the maternal plant the inflorescence was removed from the vial and a presenting anther was broken off using tweezers. The anther was then brushed onto the stigmas ensuring that pollen was deposited there. Since the inflorescences of *A. collina* are very small opticians glasses (10 x magnification) were used to ensure accurate pollinations.

In February 1989 the bags were collected and the number of capsules that had matured and seeds produced were counted.

In the spring of 1989 this experiment was repeated, but with the following changes: 10 more home plants were marked bringing the total number of home plants to 20. For each treatment five inflorescences on each of the now 20 home plants were pollinated (the total number of inflorescences per treatment therefore remained at 100) and only one inflorescence on each home plant was pollinated by each of the source plants. The 1km and 7km cross pollination distances were not repeated in the 1989 experiment.

In order to counter the predation problems experienced in 1988, in 1989 the marked maternal plants were sprayed with a commercially available systemic insecticide - Metasystox R (Bayer) - one week prior to bagging, again directly after bagging and then at regular monthly intervals throughout the experiment. Unfortunately this did not reduce the pre-hand pollination predation rate and even greater numbers of inflorescences were predated upon in 1989.

3. RESULTS

Due to the amount of predation which occurred in both 1988 and 1989 the number of replicates for each crossing distance varied greatly depending on the level of predation.

The results obtained in the 1988 and 1989 crossing experiment are given in Table 7.1 and Figures 7.1 and 7.2 respectively.

Table 7.1. The number of capsules produced per treatment. Unbagged inflorescences were marked as a control to ascertain natural pollination success and bagged inflorescences were used as a control against possible agamospermy. The other treatments are self explanatory being the distances from the maternal plant from which pollen was obtained (NN = nearest neighbour).

1988

TREATMENT	SAMPLE SIZE	CAPSULES PRODUCED	% CAPS PRODUCED
UNBAGGED	90	27	29,67
BAGGED	91	1	1.11
SELFED	96	11	10,41
NN	86	16	18.6
25M	66	11	16.66
200M	30	2	6.66
1KM	47	3	6,38
7KM	69	15	21,74

1989

TREATMENT	SAMPLE SIZE	CAPSULES PRODUCED	% CAPS PRODUCED
BAGGED	53	3	5,66
SELFED	95	15	15.79
NN	95	69	72,63
25M	70	13	18,57
200M	95	27	28,42

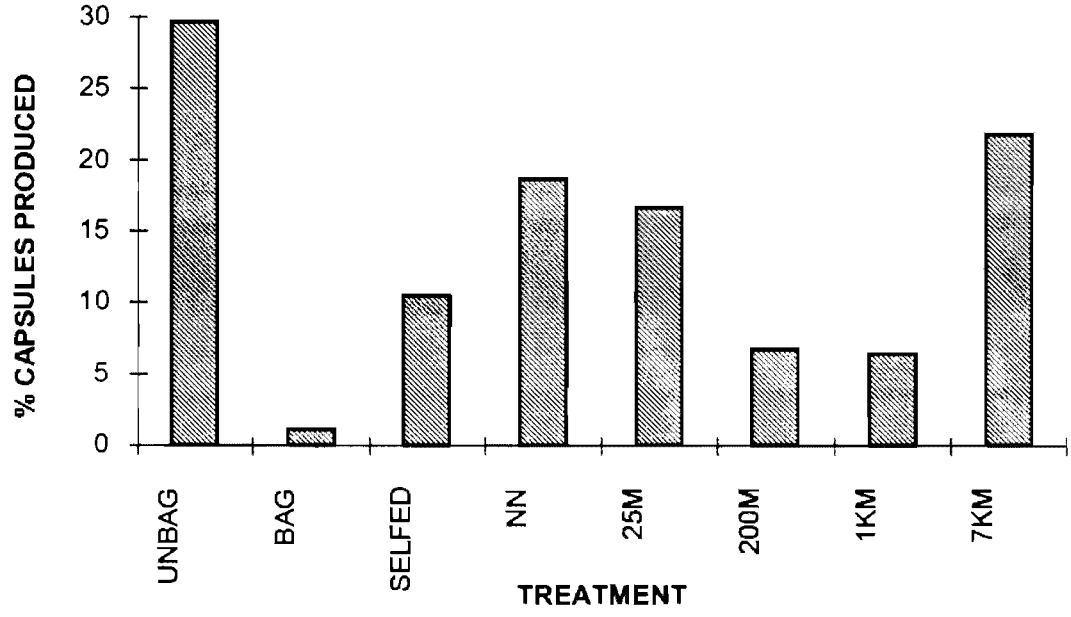

Figure 7.1. The total number of capsules produced in the 1988 cross pollination experiment expressed as a percentage of the number of inflorescences pollinated for each treatment.

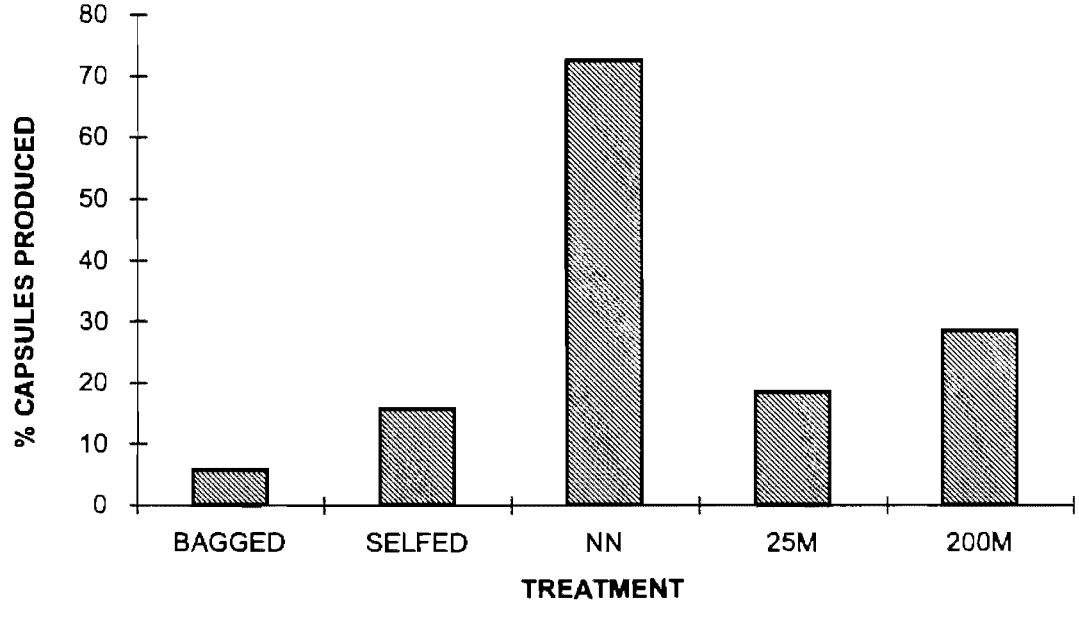

Figure 7.2. The total number of capsules produced in the 1989 cross pollination experiment expressed as a percentage of the number of inflorescences pollinated for each treatment.

An interesting fact is that the 1989 pollinations produced more capsules than the 1988 pollinations. This could have been due to optimal weather conditions perhaps but could also be as a result of the regular spraying with Metasystox which may have allowed for an increase in capsule set.

Percentage capsule set differed with different pollen donors. Very few capsules were produced from bagged plants with no pollen transfer. The bagged plants, i.e. those which were bagged from the bud stage right through the flowering season to prevent outside pollination altogether, produced 1 capsule from 90 bags in 1988 and 3 capsules from 53 bags in 1989. In both 1988 and 1989, the highest capsule set was produced from near neighbour pollinations. Lower values were recorded from geitonogamy or more distant pollen donors. When analysed statistically the data show significant differences between the successes of pollinations from nearby and from far away from the maternal plant.

The Chi Square goodness of fit test was used to compare the different pollination treatments. Expected values were calculated by dividing the total number of capsules produced for all treatments being tested by the total number of inflorescences bagged for all treatments and multiplying this by the number of inflorescences bagged for each individual treatment.

When the 1988 data is grouped into near (nearest neighbour and 25m) as opposed to distant (200m and 1 km away) pollen sources the difference found with respect to the percentage of inflorescences producing capsules was significant (Chi- Square Goodness of Fit)(Table 7.2).

In 1989 this pattern was repeated (Chi Square Goodness of Fit)(Table 7.3). The percentage of capsules set for all the treatments in 1989 was higher than in 1988.

When the selfing treatments were included in the statistical analyses the Chi-square analysis was insignificant for the 1988 data (Table 7.4) but a high level of significance was obtained for the 1989 data (the nearest neighbour treatment contributing greatly to the Chi-square) (Table 7.5).

Table 7.2. Chi-Square Goodness-of-Fit test for nearest neighbour and 25m cross pollinations vs 200m and 1km cross pollinations (1988 data).

Treatment	No. of inflorescences	Observed no. of caps produced	Expected no. of caps produced	Chi-Square
NN + 25M	152	27	21	1,6
200m + 1km	77	5	11	3,1
	229	32		

Significance level = 0.03

Table 7.3. Chi-Square Goodness-of-Fit test for nearest neighbour and 25m cross pollinations vs 200m cross pollinations (1989 data).

Treatment	No. of inflorescences	Observed no. of caps produced	Expected no. of caps produced	Chi-Square
NN + 25M	165	82	69	2,37
200m	95	27	40	4,12
	260	109		

Significance level = 0.011

Table 7.4. Chi-Square Goodness-of-Fit test for cross pollinations from selfed to 1km away (1988 data)

Treatment	No. of inflorescences	Observed no. of caps produced	Expected no. of caps produced	Chi-Square
Selfed	96	11	13	0,31
NN + 25M	152	27	20	2,45
200m + 1km	95	5	10	2,5
	343	43		

Significance level = 0.072

Table 7.5 Chi-Square Goodness-of-Fit test for cross pollinations from selfed to 200m away (1989 data)

Treatment	No. of inflorescences	Observed no. of caps produced	Expected no. of caps produced	Chi-Square
Selfed	95	15	33	9.8
NN - 25M	165	82	58	9.9
200m	95	27	33	1.1
	355	124		

Significance level < 0.01

4. DISCUSSION

Sutherland and Delph (1984) define self-compatibility as a fruit set of > 10% for self-pollinated flowers. In both the 1988 and 1989 selfing treatments the percentage of inflorescences producing capsules was just greater than 10% (11.5% and 11.6% respectively)

With regard to the breeding system of *A. collina*, therefore, the species is capable of geitonogamy according to the definition of Sutherland and Delph (1984). When the inflorescences were bagged while still in bud, however, the occurrence of pollination between flowers within the same inflorescence was very low (Figures 7.1 and 7.2). This is expected since the individuals within an inflorescence are synchronously protandrous preventing self pollination within an inflorescence. The few capsule which were produced by these bagged inflorescences are ascribed to accidental pollinations.

The results show that in 1988 the 7km treatment produced the highest percentage capsule set of all the treatments except the naturally pollinated (unbagged) treatment. Unfortunately this experiment could not be repeated in 1989 because of high predation rates on bagged inflorescences. Levin (1984) suggests that this phenomenon could be due to the relatedness of the individuals. Since the assumption throughout this experiment is that relatedness declines with distance from the maternal plant the individuals separated by 7km may be

sufficiently genetically dissimilar to result in local subdivision of genotypes. This could result in heterosis occurring in crosses from outside the normal range of gamete exchange (Redmond et. al. 1989).

The data do however clearly show that, excluding the 7km data of 1988, pollinations from nearby pollen sources were more successful than pollinations from further away (Table 7.2) with the nearest neighbour treatment producing the highest percentage of capsules. The optimal outcrossing distance as defined earlier is therefore estimated to be very short. Nearest neighbours frequently overlapped the maternal plant with the greatest distance being no more than 2m away.

These results indicate that if inbreeding depression exists in *A. collina* it is restricted to breeding within the individual and is insignificant with respect to near neighbour crosses. Outbreeding depression would probably begin between the nearest neighbour distance and 25m but no intermediate distance was studied.

Price and Waser (1979) found that the optimal outcrossing distance for *Delphinium nelsoni* was between 1 and 10m while the measured actual pollen flow was only approximately 1m. The fact that average pollen flow distances can be expected to always be less than the optimal outcrossing distance has been discussed by Waddington (1983). Waddington argues that if one takes an individual plant at the centre of a population, three zones could be distinguished around it:

- Inbreeding depression zone: That area where the individuals are so closely related to each other as a result of regular gene flow between them that inbreeding depression may occur.
- Optimal outcrossing zone: the distance from the centre of the circle where the individuals are more distantly related to those in the centre but where breeding with the central individual produces optimal success.
- Outbreeding depression zone: This zone would occur where the genetic dissimilarity between the individuals in this zone and the central individual is sufficiently great so as to result in outbreeding depression.

Logically the average gene flow distance would fall within the inbreeding zone since within this average gene flow distance the plants will by definition be most closely related to each other.

The zone of optimal genetic distance will therefore always occur further from the central individual than the average pollen flow distance in that population. Pollen flow will always be less than the optimal genetic distance, with relatively few grains moving that distance (Waddington 1983).

Based on the above, the results obtained in this experiment therefore indicate that the pollen flow distances must be very short since the optimal outcrossing distance is very short with nearest neighbour crosses producing the highest capsule set. This supports the findings in chapter 6 in which the neighbourhood area of *A. .collina*, based on gene flow, is very small.

If hand pollinated plants produce more seeds than naturally pollinated controls, then the reproductive success is being limited by pollinator activity (Bierzychudek 1981). If on the other hand reproductive success is not increased by hand pollinations then resources are presumed to limit reproductive success (Zimmerman and Aide 1989). In this experiment the highest number of capsules was produced by unbagged inflorescences indicating that capsule set in *A. collina* is probably resource limited and not pollinator limited.

5. CONCLUSIONS

The results of this experiment show that for the population of *A. collina* studied the optimal outcrossing distance is very short. This supports the results of the gene flow study in chapter 6 and suggests that most gene flow is within populations with very little gene flow between populations. In general it is expected that although the distances of optimal outcrossing and gene flow may vary between populations the trend of very limited gene flow can be expected in all populations.

Inbreeding in a population can result in a lower seed viability and seedling survival than in populations where inbreeding is minimised. A method of confirming these results would have been to germinate the seeds produced by the different treatments and to study seedling

survival (Levin 1984). A testable hypothesis therefore is that large populations, having a higher genetic diversity than small populations, may therefore produce a greater proportion of viable seeds and have a higher level of seedling survival than smaller inbreeding populations. This is therefore a possible explanation as to why the percentage seedlings increases as the population size increases as found in chapter 4.

Geitonogamous pollinations were depressed relative to near neighbour pollination, suggesting possible inbreeding depression. A method which could be used to test this hypothesis would be to germinate seed of *A. collina* obtained from populations of different sizes. The percentage germination and seedling survival could then be measured and compared. As a result of low seed set however this data was not obtainable since although the data obtained was based on capsule set, few of the capsules produced more than one seed and of the seeds that were produced, a large proportion had been destroyed as a result of predation before the bags were removed. Added to this the successful germination of *Agathosma* seed is difficult and would require further research which is beyond the scope of this study

The last word on the subject of cross pollination experiments should be in the form of three quotes from Waser and Price (1989):

- "Frustrations await those attempting to measure fitness of perennial plants in nature..."

- "..it is difficult to ensure final sample sizes sufficient for statistical demonstration of effects."

- "The effects are sufficiently subtle that it is important to look for pattern over a large sample, without which one may run a major risk of accepting a false nul hypothesis that there are no outcrossing treatment effects on seed set."

CHAPTER 8

ELECTROPHORESIS

1. INTRODUCTION

Small plant populations are subjected to inbreeding which may result in a loss of genetic diversity. Inbreeding depression may result from cross pollination between consanguineous individuals causing a reduction in viable seed set within a population and ultimately a reduction in seedling recruitment into the population. As a result of this, population growth rate is retarded. If a population is small enough or inbreeding depression is great enough the population size may even decline. It is clear, therefore, that population size can have important consequences with respect to the management of a species.

Genetic diversity is directly related to inbreeding which in turn is related to population size. A knowledge, therefore, of the genetic diversity of a population of a species or between populations of a species would be a useful management tool.

In chapter 4 it was found that there was a positive relationship between population size and seedling recruitment. In chapter 5 I attempted to find a satisfactory demographic explanation for this. In this chapter I attempted to study the genetic diversity within and between different sized populations of *A. collina* in order to provide evidence for the involvement of inbreeding and a reduction in genetic diversity in the decrease in seedling recruitment found in the different sized populations of *A. collina*. In addition the results of the cross pollination experiments of chapter 7 where the genetic relatedness of individuals could explain the differences between the different treatments could be corroborated by genetic information.

One of the most widely used procedures for revealing genetic variation in enzymes and other proteins is electrophoresis (Hartle 1988) and it is commonly used to study differences in genetic variation within and between populations. Since electrophoretic variation is subject to classical rules of Mendelian inheritance, the distribution of genotypes can be used to test for within

population events such as random mating (Hardy-Weinberg proportions) and the loss of heterozygosity or inbreeding (Grant et al 1988).

In a panmictic population the genotype frequencies present will approximate Hardy-Weinberg equilibrium. A randomly mating population is the ideal situation, however, and it is unlikely that a natural population will be panmictic. Deviations from Hardy-Weinberg resulting from positive assortative mating, selection and inbreeding can, therefore, be expected (Schaal 1975). The significance of the deviation from Hardy-Weinberg is usually tested by a Chi squared goodness of fit test and the level of deviation will give an indication of the severity of inbreeding, for example.

Wright's F-statistics are used to examine population structure at three levels, viz.; individual organisms, sub-populations and total populations (Hartle 1988, Schaal 1975, Wright 1965 in Jain and Workman 1967) and as a result there are three aspects to be considered:

- Inbreeding Coefficient (FIS).

The inbreeding coefficient is obtained by comparing the actual heterozygosity of an individual with that expected from panmixis within the subpopulation. This comparison provides an indication of the level of inbreeding.

- Fixation Index (FST).

The degree of reduction in heterozygosity of a sub-population due to random genetic drift relative to the whole population is termed the fixation index.

- Overall Inbreeding Coefficient (FIT).

This measures the reduction in heterozygosity of an individual relative to the total population.

The above indices can be used to confirm the results obtained in previous chapters of this thesis regarding the breeding system of *A. collina* as well as the level of gene flow between sub-populations of the species since the degree of heterozygosity is influenced by both of these factors. These indices when

compared for different populations and sub-populations can also be used to provide evidence of any change in heterozygosity in relation to population size.

2. METHODS

Horizontal starch gel electrophoresis was used in this analysis. In order that this part of the thesis could be carried out, an electrophoretic laboratory had to be established in the Botany Department at the University of Cape Town. This was achieved by gathering existing equipment from the department, buying it or making it myself. The Botany Department of the University of the Witwatersrand, especially Dr. E. R. Robinson, helped with advice.

2.1. Sample Collection

Actively growing shoot tips were collected from each of the marked plants in the different sized populations (see chapter 2). These samples were initially collected into small sealed vials and dropped into liquid nitrogen for storage. This method of storage was found to be unsatisfactory and after a trial period the vials were stored on crushed ice in an insulated container instead of in liquid nitrogen.

2.2. Sample Preparation

Plant samples were taken back to the laboratory and macerated within 24 hours of collection. Approximately 1g of leaves from actively growing shoot tips were ground up with a pestle and mortar in approximately 1-2ml of grinding buffer. Homogenization of the tissue by grinding disrupts cellular compartmentalization providing opportunities for interactions between proteins and secondary compounds (Kephart 1990) thus reducing enzyme activity in ground plant samples and reducing the effectiveness of the staining procedure. Since the Rutaceae contain large quantities of oils and chemical compounds this posed a problem. In an attempt to render the phenolic compounds inactive polyvinylpyrrolidone (PVP) was used in the grinding buffer. PVP bonds to phenolics forming insoluble compounds (Kephart 1990) thus reducing the interference of these phenolics with the electrophoretic process.

The material was ground as quickly as possible in cold grinding buffer to avoid excessive denaturing of enzymes and the resulting solution was poured into a vial and immediately placed on crushed ice. Two grinding buffers were used viz. Tris-HCl grinding buffer - PVP solution and Tris-Maleate grinding buffer - PVP solution (Soltis *et al* 1983). Sufficient sample was used for electrophoresis immediately and the remaining sample was frozen in a normal domestic freezer for later use.

2.3. Buffer Systems

Continuous gel and electrode buffer systems were used. The four variations used were:

Morpholine-Citrate pH 6.1 (Cheliak and Pitel 1984)

Histidine-Citrate pH 5.0 (Shields et al 1983)

Histidine-Citrate pH 5.7 (Shields et al 1983)

Tris-Citrate pH 6.3 (Soltis et al 1983)

2.4. Gel preparation (From Grant and Robinson, in prep)

A 12% starch solution was prepared as follows:
60ml cool gel buffer was mixed to a slurry with 25g hydrolysed potato starch in a 1 litre Erlenmeyer flask. At the same time 150ml of buffer was heated to boiling in a 500ml volumetric flask with a cork loosely placed over the mouth to prevent excessive evaporation of the buffer during heating.

The boiling buffer was then added to the slurry while swirling the flask continuously to prevent lumps forming in the solution. The Erlenmeyer flask containing the gel solution was then placed on the heat swirling regularly until the solution turned from an opaque viscous solution to a more translucent, liquid solution. At this point the solution was removed from the heat and degassed using a water suction vacuum pump.

The degassing process continued until all small bubbles had disappeared (±30 seconds). The solution was then poured into a perspex mould. The gel was then cooled for 10-20 minutes before covering with plastic wrap (to prevent desiccation of the gel) after which it was placed in a cold room (±4° C). Gels were usually used within 2 hours but were on rare occasions stored over night.

2.5. Gel Loading

In order to load a gel the plastic wrap was folded back sufficiently for a vertical cut to be made through the long axis of the gel approximately 3cm from the cathodal end using a sharp, clean blade. Fresh, ground plant extract was used immediately for electrophoresis where possible and the extract was then frozen for later re-using. Frozen extract was allowed to thaw at room temperature before being used after which it was immediately returned to the freezer for re-using a second time.

Since the solution was fairly viscous, centrifuging was not successful in removing solid particles from the plant extract but Whatman No. 3 filter paper wicks (3 x 7mm) were successfully used to absorb sufficient liquid extract for electrophoresis. The gel was loaded by gently pulling apart the gel at the cut and the wicks were placed on the cut surface of the anodal portion of the gel approximately 3-4mm apart. Three marker wicks containing bromophenol blue stain were loaded onto each gel in order to monitor the progress of the anodal front. The cathodal section of the gel was then pushed against the wicks and an ordinary plastic drinking straw was inserted at the cathodal end of the gel to provide pressure to ensure that the connection between the two portions of the gel was maintained and that no air bubbles were trapped in the cut.

2.6. Electrophoresis

The loaded gel was then placed across two perspex electrode buffer tanks each containing approximately 250ml of buffer solution. The plastic wrap was folded back at each end of the gel and "dailywipe" cleaning cloths were used as wicks to make contact with the buffer solution. The cloths were folded four times and pressed down gently onto the gel to ensure good contact between the buffer and the gel. The plastic wrap was then folded back over the gel and an ice pack was placed on the gel in order to ensure that the gel remained cool. Electrophoresis was carried out in a 4° C cold room to assist in keeping the gel cool. The buffer tanks were then connected to a power supply and a current was passed through the gel. After 10 minutes the current was turned off, the wicks removed and the current turned on again for 3 - 4 hours.

2.7. Slicing the Gel

After passing a current across the gel for the required time, the power was turned off and the perspex tray containing the gel was removed.

Those portions of the gel which were in contact with the cloth wicks were cut away from the gel, i.e. approximately a 15-20mm slice from both the cathodal and anodal ends of the gel. The remaining gel was then placed on a flat glass plate with a 1mm thick perspex strip glued down each side. Another piece of glass was then placed on top of the gel. The gel was sliced transversely into slabs 1mm thick by running a thin piece of nylon along the perspex strips through the gel. The gel slices were then stained for different enzymes.

2.8. Enzyme Analysis

The gel slices from both buffer systems were stained for the following enzymes using stain recipes from Soltis et al. (1983) and Cheliak and Pitel (1984).

Acid Phosphatase (APH)	Soltis et al.
Alcohol Dehydrogenase (ADH)	"
Esterase (EST)	"
Glutamate Dehydrogenase (GDH)	"
Isocitrate Dehydrogenase (IDH)	"
Malate Dehydrogenase (MDH)	"
Malic Enzyme (ME)	"
Shikimic Acid Dehydrogenase (SDH)	"
6-Phosphogluconate Dehydrogenase (6PGD)	"
Peroxidase (PER)	Cheliak and Pitel

All gel slices were stained using baths containing 100ml of staining mixture.

Once staining was complete the gels were fixed in a 5 Methanol : 5 Distilled Water : 1 Galacial Acetic Acid solution.

3. RESULTS

Of the 10 enzymes tested only MDH gave some unclear results and then only irregularly. Only horticultural samples of *A. collina* showed promise of significant results.

4. DISCUSSION

As a result of problems with technique and chemical compounds in the plant tissue very little electrophoretic data was obtained from *A. collina*. Some of the identified problems are discussed.

Initially the plant samples collected in the field were stored in liquid nitrogen and at times no enzymatic activity was detected during electrophoresis. In a paper published while this experiment was in progress Kephardt (1990), in her analysis of methods used, found that respondents rated frozen plant material as the least preferred method of storage before grinding and some respondents reported a loss of enzyme activity after freezing at temperatures below -20 C. Plant samples were therefore collected onto crushed ice in an attempt to maximize enzyme activity during electrophoretic staining. Although this resulted in some staining the results were seldom interpretable.

Since there were high levels of chemical compounds in the leaves of *A. collina* the Tris-Maleate grinding buffer - PVP solution of Soltis et al. (1983) was initially used since it contained sodium tetraborate, sodium metabisulphite, diethyldithiocarbamic acid and ascorbic acid as well as polyvinylpyrrolidone (PVP) all of which act to reduce the reaction of these compounds with proteins which causes a reduction of the enzyme activity during the electrophoretic process (Kephardt 1990). Despite the use of this grinding buffer, however, browning of the plant tissue extract frequently occurred providing a visual guide to the possible loss of enzymatic activity from phenoloxidase products (Kephardt 1990). The Tris-HCl grinding Buffer - PVP solution of Soltis et al. (1983) was then used and the problem of the browning of the extract did not re-occur.

Samples from an *A. collina* plant taken from a garden in the Cape Town area were also used during the initial electrophoresis experimentation. These samples invariable showed stronger bands after staining but the results were still inconsistent. These individuals were well watered and were growing in a more fertile soil than those from the study site. These results indicate that the plants from the study site may have been more stressed and as a result their enzyme activity was less than those from the Cape Town garden resulting in problematic protein extraction and staining. The inconsistencies in the results found in both the plants from the study site and the garden in Cape Town indicate a problem with electrophoretic technique.

Without electrophoretic results it is unfortunately not possible to discuss differences in heterozygosity, gene flow or the breeding systems of *A. collina* with respect to electrophoresis. This is unfortunate since such information could provide confirmation of the results and hypotheses of previous chapters in this thesis. Comparisons of the amount of variation within and between different sized populations may have assisted in explaining the relationship between population size and seedling recruitment found in Chapter 4. Electrophoretic information may also have supplied additional information supporting the cross pollination results of Chapter 7 where the genetic relatedness of individuals could have explained the differences in the number of successful pollinations between the different treatments. With further experimentation however it may be possible to obtain useful results from electrophoresis of *A. collina*.

CHAPTER 9

CONCLUSION

In chapter one I set out six key questions which I would have liked to have answered by the end of this thesis. In order to conclude this thesis I will take each question which was initially posed and discuss what has been discovered.

1. What is the breeding system of *A. collina*?

A. collina is both andromonoecious and protandrous and the species is insect pollinated. The flowers of each inflorescence are synchronous with respect to the times at which they are staminate or pistillate but neighbouring inflorescences may be at different stages. *A. collina* is geitonogamous according to the definition of Sutherland and Delph (1984) because the percentage of self pollinated inflorescences producing capsules was >10%, but the percentage capsule production was only 11,5% and 11,6% for the two years.

The number of inflorescences on *A. collina* plants were at their lowest in June with peak flowering occurring around October/November. In addition the number of flowers per inflorescence was low outside the peak flowering season.

Male/hermaphrodite ratios of the inflorescences were highest in June while at peak flowering time the M/H ratios were much reduced.

2. How does population size affect demography?

In chapter four I set out to discover one basic fact: was there any evidence that population size had an effect on population structure? In this study I found that larger populations had a higher proportion of seedlings and that fewer seedlings were recruited by small populations. This indicates that there was a probability that a minimum viable population size may have indeed existed for this species.

The demographic studies carried out to explain this pattern were, on their own, inconclusive. No single aspect of the demography of *A. collina* indicated a consistent population size effect each year. Frequently trends which seemed to be there were not significant. Increasing the sample size would possibly clarify these trends but for much of the information collected this would not be practical. The number of years over which the study was carried out could, however, be increased. This would help counter possible climatic differences between the years which may have affected the results. In

addition, since the laboratory germination of the seeds of *A. collina* was difficult, seed germination and seedling survival were not studied. If germination of *A. collina* seeds under controlled conditions could be consistently achieved then seed germinations, seedling survival and seedling growth data could add valuable information to that already obtained.

3. Is there a difference in reproductive output in different sized populations?
The answer to this question is a simple yes. Chapter four showed that there were more seedlings in larger populations than in small. In chapter five I studied different aspects of demography which could have caused this result.

Two possible demographic aspects which could have contributed to this were inflorescence predation and seed set per capsule. The predation data was collected over one flowering season only and although it was shown that population size and inflorescence predation could be correlated in that medium sized populations suffered more predation than small and large populations, these results did not correlate with information from chapter four. The predator satiation argument of Janzen (1971) provides the most likely explanation for these results.

In terms of seed set per capsule, however, significant results were obtained in 1988 indicating a correlation with population size. The 1989 data, however, were not significant at all. Since this sort of information is relatively easy to gather, more representative results could perhaps be obtained over a longer period of time, say five years to help counter climatic fluctuations, stress levels, pollinator activity or even predation levels.

Ecological consequences of small populations include reduced seed viability, germination rates and seedling growth rates, which were not tested in this thesis, and while small populations carry some ecological risks much emphasis in the conservation literature has been placed on genetic risks.

Since the population size effects identified above could not be satisfactorily explained through demography it was necessary to test the assumption made, when selecting

populations of *A. collina* for this study, that the populations (or sub-populations) studied were genetically isolated from each other. If it could be shown that the populations were isolated then genetic effects (e.g. selfing and genetic drift) may well have contributed to the demographic problems identified. Inbreeding depression and loss of genetic variation may follow as a result of genetic drift or increased self pollination in a small population hence the following two key questions.

4. What is the gene flow pattern within and between populations of *A. collina*?
Accurate gene flow data proved difficult to obtain (chapter 6). Pollen dispersal experiments using fluorescent dye as a marker were not successful and myrmecochorous dispersal of seed was not observed. Basic assumptions were made and data from other sources was used to get some indication of what the situation may have been. Results which were obtained in chapter six indicate that gene flow may be very limited.

5. Is there an optimal genetic distance from which pollen should come which would increase seed set over pollen from any other distance?
Chapter seven set out to directly answer this question. Pollinations from extremely far away, 7km, produced the highest percentage capsule set of all treatments, but this sort of pollen movement would be highly unusual. Within the realms of what could naturally be expected, pollen transfer between nearest neighbours provided the highest capsule set. This result corroborates the results obtained from the gene flow experiments which indicated that gene flow may be very short in this species.

To improve upon the results of this work, an option would be to collect plants from their natural locations and pot them. This experiment could then be carried out in a glass house. Since the experiment was carried out outside, climate and other factors may have affected the results and certainly made the work more difficult. Predation of inflorescences prior to pollination would also be able to be better controlled in a protected environment.

6. Is there a difference in genetic diversity in different sized populations?

Unfortunately the electrophoretic experiments were not successful but with further experimentation the secrets of *A. collina* electrophoretic methods should be possible to solve. The results obtained from horticultural samples of *A. collina* indicate that well watered and protected plants of *A. collina* will give more consistent results than those collected from their natural situations.

If therefore, as has been proposed above, plants are potted and grown in a glass house, electrophoretic techniques may yield better results. With a lot of field data already collected further electrophoretic experiments of this sort may prove extremely useful.

In summary, therefore, this study used a relatively common but localized species to assess population size effects. If significant population size effects were detected this would indicate concern for other threatened taxa. What the study showed was:

1. Small population size effects do exist as predicted by theory.

2. It is possible that the cause of these effects are genetic rather than ecological.

3. Genetic effects are most likely where species are self compatible and where gene flow distances by both seeds and pollen are very short.

4. The population size effects were limited and only identified in very small populations.

What this thesis indicates is that genetic problems arising from small population size may be real but the population sizes at which these problems occur may only be in unusually and atypically small populations.

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