CHAPTER 5

CONCLUSIONS

The findings of the study show that the populations of *Viscum capense* and *Viscum rotundifolium* at Makapansgat are both relatively small and probably originate from very few founder individuals. While *Viscum capense* in this area infests 5 different host species, *Viscum rotundifolium* is found on only one host species although it infests various other hosts in other areas. Information gathered on the pollination of these species shows that both species are pollinated by flies that do not collect pollen, so they are presumably attracted to these flowers by nectar production of some sort. The hypothesis for pollinator attraction to large pollen crops in dioecious species, therefore, does not apply to *Viscum capense*.

Although different species of flies are likely to be the major pollinators for each species, the end result in terms of gene flow seems to be similar. The distance over which pollen is likely to be transferred is small, in the case of each of these pollinators. Thus, even in the dioecious species, most pollination events are likely to take place between plants in the same tree. Due to the behaviour of the bird species in the area, a large percentage of seeds is dispersed within the same tree as the female parent. As a result of this, pollination events will most often take place between parent plants and their offspring, as well as between half and full siblings. For *Viscum capense* even the crosses between half siblings may not result in the formation of many new gene combinations, since the presence of translocation rings limits recombination and promotes linkage. This problem is exacerbated by a high degree of clumping in both species, which is particularly marked in the monoecious species which exhibited extreme clumping with a mean plant to plant distance of 8 cm. The results of this study show that monoecious species are likely to be more prone to clumping than the dioecious species. As a result of clumping in both species, the pollinators and dispersers are presented with high rewards in small areas and gene flow is limited, probably resulting in high levels of inbreeding, even in the dioecious species. Since the size of the genetic neighbourhood in both species is likely to be small, the dominant evolutionary forces which shape the distribution of genetic variation for these species may even be chance events, rather than selective processes.

As regards the evolution of dioecy, the results of the ecological section show no evidence for disruptive selection causing spatial separation of males and females into microhabitats where

they perform best. Thus the hypothesis for the evolution of dioecy through selection for minimising intersexual or intraspecific competition (Freeman et al 1976), can be excluded in this case.

The pollen to ovule ratio calculated for each species shows that the shift to dioecy may also have some costs. In order to achieve a percentage seed set 10% lower than that of the monoecious species, the males of *Viscum capense* have to produce five times as much pollen as the male flowers of *Viscum rotundifolium*. Even if the females of *Viscum capense*, on average, had more flowers per plant than *Viscum rotundifolium*, only half of the plants in their population would bear seed, therefore they would have to double the amount of flowers per plant, and then have another 10% more flowers just to equal the reproductive success of the monoecious species. Not only was the monoecious species saving in the amount of pollen it needed to produce, but fewer resources were required to produce male flowers since the ratio of male to female flowers in *Viscum rotundifolium* was 1:2 compared with 1:1 in *Viscum capense*.

The hypothesis that unisexual males would be more attractive to pollinators due to the large pollen crops (Opler & Bawa 1978), falls away in this study since the pollinators of *Viscum capense* do not collect pollen. Disperser attraction to larger fruit crops on unisexual female plants is not as clear cut, since there is no data on the actual number of female flowers per plant in both species due to the reasons given in the section on materials and methods. Since the seed-bearing plants of *Viscum rotundifolium* are much more clumped than those of *Viscum capense*, the monoecious plants in fruit may actually be more attractive to the dispersers.

The distribution of seed-bearing plants has other implications however in the light of seed predation. In terms of Janzen's hypothesis (1971), the dioecious species may have an advantage in terms of escaping seed predation, since its seed-bearing plants do have a less clumped distribution than the seed bearing plants of *Viscum rotundifolium*. Whether or not a lesser degree of clumping in *Viscum* is advantageous in terms of seed predation is uncertain, since no seed predators were observed during this study.

Perhaps the most convincing data for refuting any of the hypotheses comes from the genetic section. These results showed no significant difference in the levels of heterozygosity between the monoecious and the dioecious species. If the monoecious species can maintain similar levels of heterozygosity to the dioecious species, it is doubtful whether dioecy would have been

established through selection for outcrossing, since the presumed benefits of outcrossing are prevention of inbreeding depression and an increase in heterozygosity.

The genetic data also indicates some mechanism operating in these species of *Viscum* which is responsible for maintaining what may well be a large proportion of the genome in a heterozygous state. Agamospermy is, unlikely, at least in the dioecious species, due to the population sex ratio, so it must be assumed that sexual reproduction is taking place. More research will have to be undertaken to determine what this mechanism for maintaining genetic heterozygosity is.

To return to the evolution of dioecy then, selection for outcrossing and several of the proposed hypotheses, have been eliminated. The major differences between the monoecious and dioecious species studied lie in the pollen to ovule ratio, the degree of clumping of the population, the delay of fruit ripening in the dioecious species and the presence of translocation complexes in only the dioecious species. The question still remains as to whether any of these factors could have been a selective advantage of dioecy.

If dioecy had been introduced into the population through translocations leading to chromosome rings (Wiens & Barlow 1979), the first dioecious plants would have a disadvantage in that 50% of their pollen would be inviable (Garber 1972), due to adjacent chromosomes migrating to the same pole. The establishment of a directed or zig-zag orientation at meiosis may take some time, thus the dioecious plants are likely to have had some disadvantages.

The differences in the clumping of populations was shown to be a consequence of breeding systems, due to the tendency of all seed-bearing plants to accumulate more plants within the same tree as themselves. Thus any advantages of reduced clumping could be a selective pressure for the establishment of separate sexes. The lack of seed predators and potential advantage of clumped seed-bearing plants in terms of disperser attraction however complicate the issue since there is a potential conflict between disperser attraction and avoidance of predation.

The quality of seeds and thus the improved prospects for establishment of dioecious plants may be an important selective advantage in *Viscum capense* due to their ability to postpone ripening of fruits and perhaps maximise input into seeds every second season.

There does not appear to be any one major selective advantage of dioecy in terms of the range

of attributes examined in the study. Why then did dioecy evolve in *Viscum*? Assuming that Wiens and Barlow (1979) are correct in their hypothesis that dioecy was established by the linkage of factors for maleness and femaleness in translocation complexes (found in dioecious species only), when this occurred, there must have been immediate perfect n[illegible] and females and no intermediate individuals with flowers mainly of one sex should have occ[illegible]red.

As a result of the translocations responsible for this condition, the chromosome structure of the dioecious mutants would be rearranged and thus could well be incompatible with t[illegible] untranslocated chromosomes of the monecious plants. It is this very process which has led other workers to hypothesize rapid speciation in the genus *Clarkia* which also exhibits translocation heterozygosity (Lewis & Raven 1958). Since chromosomal reorganisation can rapidly result in reproductive isolating barriers and thus, in rapid speciation (Gottlieb 1973, Lewis & Raven 1958 and Walbot & Cullis 1985), it means that if dioecy is fixed purely by the linkage of sex factors through translocations (Wiens & Barlow 1979), rapid speciation leading to a dioecious species most probably took place in *Viscum* as well.

The dioecious plants, which had similar translocations, would be compatible, but they would most likely not be compatible with the untranslocated monoecious plants. If this was the case, there is no need to invoke any selective advantage for the establishment of a dioecious species, since any crosses between this and the monoecious species would be inviable. In this way there is no need for the dioecious plants to be superior in any way in order to eliminate the "genes" for monoecy from their genome. As long as they were able to reproduce themselves, they would survive as a species and never produce any monoecious plants unless further translocations occurred which freed the sex factors from linkage. If this did occur, however, it would most probably constitute another speciation event. Thus it seems possible in *Viscum*, that dioecy could have evolved in the absence of selection for separate sexes.

Whether or not this particular case has any bearing on dioecy as a whole is debatable. Many examples have been found in which dioecious species are shown to have distinctive advantages over hermaphrodites, so undoubtedly in some cases dioecy must have been selected for.

The distribution of dioecy in the angiosperms, as mentioned in the introduction, shows, however, that dioecy never dominates high taxonomic categories in the angiosperms (Richards 1986). This implies limited evolutionary potential and perhaps, therefore, few selective advantages in

plants. At the same time, dioecy crops up in many different lineages (Yampolsky & Yampolsky 1922 in Bawa 1980). It can be hypothesized that instead of this situation being an evolutionary consequence of selection for separate sexes, it could be a consequence of mutations involving the diverse sex determination mechanisms, which result in reproductive barriers, thus establishing a dioecious species of sometimes limited selective advantage. This of course raises another question, as to whether evolution can proceed, not only through natural selection favouring the fittest individuals in a population, but also through survival of other mutants which escape elimination of their characteristics from a gene pool by speciating rapidly through erection of reproductive barriers.

CHAPTER 6

ASPECTS FOR FURTHER RESEARCH

As with most projects, this investigation raised more questions than it provided answers. The biology of the two species of *Viscum* studied proved most interesting and, in some cases, highly unusual. Some of the areas which should yield interesting results if pursued, are listed below.

Resource allocation remains one of the major hypotheses which was not tested adequately in this study. If tests could be devised which would overcome the problems set out in the materials and methods, the results may prove interesting.

A test could be formulated to determine whether *Viscum capense* ssp *capense* actually does maximise investment to its seeds as a result of postponing ripening. A number of female plants could be labelled at the onset of a flowering season and the numbers of flower buds for the present season, and ripe fruits from the previous season, should be counted. If data showed that the plants which had large fruit crops from successful pollinations the previous year, in general had fewer flower buds for the current season than the plants with few fruits, it would indicate preferential allocation to fruit and seed development, rather than to an additional reproductive effort in terms of flowering. Thus, *Viscum capense* could be said to be maximising the quality and not the quantity of its reproductive output. Examinations of other monoecious and dioecious species should be made to determine whether this trend is a characteristic of both monoecious and dioecious species or whether it is only present in the dioecious species.

Other tests could be made using plants that show similar numbers of unripe fruits at the end of the flowering season. The fruits from some of these plants could all be removed, and in the following flowering season, the numbers of flowers on the plants which had their fruits removed should be compared with the number of flowers found on the plants whose fruits were left intact. Thus it could be shown whether resources which would normally be allocated to fruit development, could be reallocated to flowering, if there was a difference in the numbers of flowers produced by each of these types of plants.

Electrophoresis of isozymes could be used for a wealth of investigations into these species, and even in this study, showed its worth in testing taxonomic relationships (confirming the close

relationship between the two subspecies of *Viscum capense*). A study of phylogeny in the genus *Viscum* is possible, and a lower level of genetic resolution than was required for this study would be acceptable, since the species of *Viscum* appeared to be so different genetically, that it would be sufficient to determine the number of loci the species have in common, rather that the number of alleles. Information on the phylogeny of these species would be extremely interesting, since it could possibly show whether dioecy had evolved only once in this genus or whether it had numerous independent origins, depending on the relationships between the monoecious and dioecious species.

Electrophoresis, in conjunction with breeding studies, could be used to investigate further the mechanism which is responsible for maintaining heterozygosity at so many of the loci in these two study species, by observing the inheritance of alleles in controlled crosses. It could also be used to determine whether or not these loci are characteristic of all species of *Viscum*.

Isozyme work is possible using the embryos of the species of *Viscum*. This was tested at one stage in this investigation and it was found that the best method was to crush individual embryos directly on to filter paper wicks soaked in the extraction buffer and insert these into the gel. This method was adopted after reading Soltis' method for fern gametophytes (Soltis et al 1983). A collection of a large number of fruits from one female plant or hermaphrodite could be analysed for the loci which have been shown to exhibit variability (PRX 3 & ME 1 or 2). If the female parent was also tested for this locus, it could be shown whether more than one male plant was responsible for pollinating that female, particularly for loci which have more than two alleles. It would also be a test for agamospermy and could show whether or not the embryos which develop from a single seed are derived from the same male parent. The latter information would be extremely important in terms of colonisation, since a single seed being dispersed even of the dioecious species could give rise to a population and start with more than two alleles at some loci.

Some of the loci which are fixed for heterozygosity should also be checked in the embryos, since if the genetic situation in the adults is due to strong selection against homozygotes in the establishment of seedlings, some homozygotes would be present in the embryos. Isozyme electrophoresis could therefore be used to test this hypothesis effectively.

Finally, an extensive cytological survey is needed in order to confirm Wiens and Barlow's findings

of the lack of translocation heterozygosity in monoecious species. The survey should also investigate the possibility of other meiotic abnormalities which may be responsible for some loci exhibiting recombination and others not. The genus *Viscum* appears to be an extremely unusual group of species, and as a result, it is likely that any investigations into the biology of these species would be rewarded with extremely interesting results.

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APPENDIX

EXTRACTION BUFFERS

Cheliak and Pital grinding buffer

To prepare 100ml of buffer:

8% w/v (8.0g) PVP

0.3M (10.26g) sucrose

0.5mM (0.015g) EDTA

1.0mM (0.015g) dithiothreitol

1.0mM (0.018g) ascorbic acid

0.1% (0.1g) bovine serum albumin

0.4mM (0.26g) NAD

0.3mM (0.24g) NADP

0.2mM (0.5g) pyrodoxal-5-phosphate

0.66M 2-mercaptoethanol

Adjust to pH 6.7 with 1M Tris.

(Cheliak and Pitel 1984)

Phosphate grinding buffer - PVP solution.

To prepare 25ml of buffer:

0.029M (0.28g) sodium tetraborate

0.017M (0.08g) sodium metabisulphate

0.2M (1.0g) L-ascorbic acid sodium salt

0.016M (0.07g) diethyldithiocarbamic acid sodium salt

4% w/v (1.0g) PVP average molecular weight 40 000

Add above to 25ml of 0.1M phosphate buffer pH 7.5.

To prepare 100ml of 0.1M phosphate buffer:

1.36g KH PO

9.0ml 1M NaOH

0.25ml (1%) 2-mercaptoethanol

Make up above to 100ml with distilled water and pH with NaOH.

(Soltis et al 1983).

Tris-HCl grinding buffer - PVP solution.

To prepare 25ml of buffer:

0.001M (0.01g) EDTA (tetrazolium salt)

0.01M (0.019g) potassium chloride

0.01M (0.05g) magnesium chloride hexahydrate

0.25ml (1%) 2-mercaptoethanol

4% w/v (1.0g) PVP average molecular weight 40 000

Add above to 25ml of 0.1M Tris-HCl buffer pH 7.5.

To prepare 100ml of Tris-HCl buffer:

0.1M (1.2g) Tris

100ml distilled water

pH with HCl.

(Soltis et al 1983).

Tris-maleate grinding buffer -PVP solution.

0.2M (1.91g) sodium tetraborate

0.02M (0.095g) sodium metabisulphate

0.25M (1.24g) L-ascorbic acid sodium salt

0.026M (0.11g) diethyldithiocarbamic acid sodium salt

0.1M (0.29g) maleic acid

0.1M (0.3g) Tris

0.25ml (1%) 2-mercaptoethanol

4% w/v (1.0g) PVP average molecular weight 40 000

Add to 25ml distilled water

pH with 1M HCl.

(Soltis et al 1983).

RUNNING AND GEL BUFFERS

All gram quantities given for preparation of 1 litre of buffer.

Tris-citrate pH 7.5

Electrode buffer:

0.223M (27.00g) Tris

0.086M (16.52g) citric acid

Gel buffer:

Dilute 35ml of electrode buffer to 1 litre.

(Soltis et al 1983).

Tris EDTA borate pH 8.6

Electrode buffer:

0.18M (21.8g) Tris

0.004M (1.52g) EDTA

0.1M (6.18g) boric acid

Gel buffer:

Dilute 250ml of electrode buffer to 1 litre.

(Soltis et al 1983).

Morpholine-citrate pH 6.1

Electrode buffer:

0.04M (7.684g) citric acid

Adjust to pH with N-(3-aminopropyl)-morpholine

Gel buffer:

Dilute 50ml of electrode buffer to 1 litre.

(Shields et al 1983).