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The consequences of monoecy and dioecy in congeneric species, and their implications for the evolution of dioecy in the genus Viscum

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2015-02-05
http://hdl.handle.net/10539/16899

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CHAPTER 4

DISCUSSION

All of the data collected in this study are useful for obtaining an overall picture of the ecology, reproductive biology and genetic structure in both the monoecious and dioecious species of *Viscum*. Since the same information was collected for both species, it is possible for some comparisons to be made between these features of each species. Where differences exist, the question can be asked as to whether these differences are a direct result of the breeding systems, or if they could be explained in some other way. If it seems likely that the species differ in any way as a result of their alternative breeding systems, the evolutionary consequences of these differences can be discussed. Since the nature of the study was to detect differences which would have evolutionary consequences, particularly those differences which would give dioecious plants an evolutionary advantage, much of the data can be used to test specific hypotheses for the evolution of dioecy.

The general findings will be discussed and then their implications for the evolution of dioecy will be dealt with.

ECOLOGICAL DATA

The population of *Viscum capense* ssp *capense* at Makapansgat was found to be small, consisting of only 83 plants, with a sex ratio of male to female plants not differing significantly from 1:1. It was found that although the plants were able to infest 5 different tree species in this area, they were not randomly distributed, but were highly clumped. Although this would be expected to a certain extent, due to the plant's parasitic lifestyle limiting them to available hosts, the results are still significant since the average number of plants per host is 2.45. This shows that although the population is clumped, there are few trees with a large number of plants contained within them, instead, there are only 4 host trees out of 31 which have more than 3 plants growing in them. The fact that the population is clumped, in spite of the relatively low numbers of plants per tree, indicates that either the host trees themselves were clumped, or that dispersal does not take place over large distances or in a random fashion. A high number of plants per tree could have raised the degree of clumping of this species considerably, but since 18 out of the 31 host trees had only one plant growing in them, a large proportion of the nearest

neighbour measurements were actually measurements between plants in different trees. The clumped distribution has obvious connotations for gene flow and dispersal and this will be discussed further in the sections on reproduction and genetics.

The population of *Viscum rotundifolium* at Makapansgat was also small, consisting of only 35 individuals. Similar small populations were also noted at Nylsvley, Grahamstown and in the Magaliesberg where one population consisted of only 13 plants. Unlike *Viscum capense*, *Viscum rotundifolium* only infested one species at Makapansgat, but whether or not this is of any significance is indeterminable since *Viscum rotundifolium* is the most common and widespread species of *Viscum* in South Africa and occurs on a large number of different hosts in different areas (Wiens & Tolken 1979).

Viscum rotundifolium at Makapansgat is also highly clumped, but much more so than *Viscum capense*. Although *Viscum rotundifolium* infests only one host species in the area compared to the five host species of *Viscum capense*, it is quite possible that other species of potential hosts are present in the valley since *Viscum rotundifolium* is known to infest a large number of diverse hosts (Wiens & Tolken 1979). One contributing factor to the high degree of clumping in this species, may be that the host trees of *Viscum rotundifolium* (*Ehretia rigida*) are themselves clumped within the area, with an R value = 0.171, the same as the R value for the clumping of *Viscum capense*. The high density of plants per host (mean no. = 11.67) and the low mean plant-to-plant distance (0.08m) however, still indicate a higher degree of clumping in this species. The high density of plants within the host trees of this species accounts entirely for the clumping of this species, since the nearest neighbour for each plant of this species was another plant growing in the same tree. This means that the high degree of clumping of *Viscum rotundifolium* plants is independent of the degree of clumping of its' host species.

In addition to determining the degree of clumping, the nearest neighbour analyses provide further data which indicates the unlikelihood of disruptive selection operating in the dioecious population to enable the species to maximise the available resources and minimise intraspecific competition. The mean plant-to-plant distance for *Viscum capense* is 2.23m (s = 4.89) and the mean male-to-male, female-to-female and male-to-female distances are 3.20m (s = 7.95m), 1.34m (s = 2.28m) and 1.55m (s = 3.48m) respectively. Had the mean male-to-male and female-to-female distances been less than the mean plant-to-plant or male-to-female distances, it would have indicated the possibility of preferential sites or hosts for each sex which could mean that disruptive

selection had taken place.

The mean female-to-male and female-to-female distances are not significantly different when the standard deviation is considered, indicating no spatial segregation of sexes and, therefore, no evidence for disruptive selection. To further the case against disruptive selection, the population distribution shows that 69% of the plants occur in trees in which both sexes are present.

The large male-to-male distance (3.20m) relative to the female-to-female distance (1.34m) and male-to-female distance (1.55), is easily explained since the presence of one female plant in a tree greatly increases the likelihood of further plants occurring in that tree in subsequent seasons due to fruit production by that female. The same is obviously not true for male plants.

REPRODUCTIVE DATA

The pollen to ovule ratio of *Viscum capense* is 12012:1, an extremely high value. Table 4.1 shows Cruden's values calculated from average counts from plants with various breeding systems giving pollen to ovule ratios typical of different breeding systems (Cruden 1977).

BREEDING SYSTEM	POLLEN TO OVULE RATIO
Cleistogamy	4.7:1
Obligate autogamy	27.7:1
Facultative autogamy	168.5:1
Facultative xenogamy	796.6:1
Xenogamy	5859.2:1

(After Cruden 1977)

A value of 12012 is therefore indicative of an obligate outcrosser, which *Viscum capense* subspecies *capense* undoubtedly is. It also suggests inefficient pollen transfer since such a large

number of pollen grains are required to achieve fertilization of a single 'ovule', similar to the situation one would expect in a wind pollinated plant. Pollen transfer does however take place to the extent that 47% of the female flowers set seed. From the numerous visitors to the flowers of this species and from the structure of the female flower (plate 1.2), it seems highly u[illegible]y that this species is wind pollinated. It seems far more likely that flies are the principal pollinators, and that they are attracted to the flowers by the nectar production of b[illegible]nales and females. Thrips and ants may be of some importance in transferring pollen as well, where the male and female plants grow in close association. Even though some flies (especially the horseflies) are powerful fliers (Borror & DeLong 1964), the close association of plants makes it seem likely that pollen transfer would often not take place over large distances, since the flies tended to clean themselves of pollen quite regularly.

The pollen to ovule ratio for *Viscum rotundifolium* is 2148:1 which puts it between Crudens' categories of facultative xenogamy and xenogamy. In contrast to *Viscum capense*, it presumably has the ability to self (unless it possesses a self-incompatibility system), since mature male and female flowers can be found at the same time on any given plant, even though the male flowers of individual cymules usually release pollen before the two lateral female flower buds open. Assuming that selfing is possible, this should place it somewhere between facultative autogamy and facultative xenogamy, thus its' pollen to ovule ratio is higher than one would have expected from its' breeding system. This pollen to ovule ratio which is considerably lower than the ratio obtained for *Viscum capense*, still results in 56% of the female flowers setting seed.

Although the major pollinators of *Viscum rotundifolium* appear to be midges or fruitflies, instead of carrion and horseflies, it still seems highly likely that pollination in these species still takes place over short distances, due to the extreme clumping of these plants, which provides the pollinators with ample rewards in a small area. Even if pollen carry-over does occur (Levin 1981), the large number of flowers in a single tree may well mean that pollen will most often be transferred to to a[illegible]other plant in the same tree, or even another another flower on the same plant.

The shift to dioecy has obviously resulted in a large increase in pollen production (5 x greater in *Viscum capense*), but the hypothesis which states that large pollen crops could be a selective advantage of dioecy in terms of attracting pollinators, most probably does not apply here, since as flies are the pollinators, pollen is unlikely to be the attractant in either species. Since pollen is probably not acting as an attractant, its only purpose must be to achieve fertilization. Thus the

large amount of pollen produced by the dioecious species could be seen as a cost of dioecy, since it requires a much larger amount of pollen to effect a lower percentage seed set than that obtained in the monoecious species.

In this shift to dioecy, the separation of sexes has led to an increase in resource allocation to the male function, which in turn did not lead to an increase in percentage seed set, but rather a decrease due to the greater distances over which pollen had to be transferred. In order for this situation to be of greater selective advantage than the situation in the monoecious species, the reduction in percentage seed set would have to be compensated for by an increase in the quality or the vigour of the plants produced by outcrossing.

If a shift to dioecy was accomplished by translocation heterozygosity as proposed by Wiens and Barlow (1979), the early dioecious species would have experienced high levels of pollen abortion. Until a zig-zag orientation of translocation complexes across the equator was established for, 50% of a plant's pollen would be inviable due to co-migration of adjacent chromosomes in the complexes (Garber 1972). Although this situation would not be a direct disadvantage of dioecy, it would still be a disadvantage associated with all early dioecious species. In time, the orientation of complexes can become directed (form a zig-zag) and result in higher levels of pollen viability (Garber 1972). In *Viscum capense*, only 6.1% of the pollen grains were inviable, so directionality must be strong in this species. Strangely enough, 20.8% of the pollen grains of *Viscum rotundifolium* were inviable, even though there are no reports of translocation heterozygosity in this species (Wiens & Barlow 1979).

Female plants in the population of *Viscum capense* tend to be clumped, with an R value of 0.069 and since 47% of all the female flowers set seed, large seed crops are presented for disperser attraction, an assumed advantage of dioecy (Opler & Bawa 1978). At the same time, an aggregation of seed-bearing plants is potentially disadvantageous in terms of seed predation (Janzen 1971), where a more spread out distribution of seed plants is assumed to make it more difficult for seeds to be located. In this light, the relationship between attraction of dispersers and avoidance of predation appears to be antagonistic. *Viscum rotundifolium* has a percentage seed set 10% higher than that of *Viscum capense* and the seed-bearing plants of the former are certainly more highly clumped (R = 0.00037) than those of the dioecious species (R = 0.069). This could then be construed as a potential advantage of dioecy in the light of Janzen's hypothesis (Janzen 1971). In Janzen's view, two consequences of dioecy are an increased seed set and an

increase in distance between seed-bearing plants, since only half of the plants would bear seed. Both of these consequences he believes would aid in escaping seed predation and thus they are selective advantages of dioecy (Janzen 1971). While there is no data for testing the first of these assumed consequences, the above results do show a lesser degree of clumping of seed-bearing plants in the dioecious population. One must remember however, that the female plants of *Viscum capense* are not only less clumped within the area because they represent just half of the dioecious population, but the dioecious population, as a whole, was far less clumped than the monoecious population (R = 0.17 and R = 0.00037 respectively) and it is as a result of both these situations that the females of *Viscum capense* are much less clumped than the seed-bearing plants of *Viscum rotundifolium*. The distribution of the females therefore cannot only be ascribed to their being half of the dioecious population, which obscures the validity of Janzen's hypothesis in this case. This discussion will continue later in the light of further data.

The degree of clumping of the seed-bearing plants in the dioecious population is nonetheless intriguing, since in contrast to Janzen's prediction, the female plants (R = 0.058) were more highly clumped than the dioecious population as a whole (R = 0.17). This result is a function of using Clark and Evans' (1954) method for calculating aggregation. The number of plants in the population drops to approximately half when the males are removed but the area over which the plants occur, remains the same. Thus, when calculating the expected distance between plants in the area, the distance works out as being approximately twice the distance one would expect for the total population. Since the mean female-to-female distance in the population does not differ significantly from the mean plant-to-plant distance, the degree of clumping of female plants then works out to be approximately twice as great as the degree of clumping of the whole population. The high degree of clumping of the female plants is therefore an artefact of Clark and Evans' (1954) method. The mean distances between seed-bearing plants are probably more meaningful measures of aggregation in this case.

The above situation raises some questions about Janzens' hypothesis that the distribution of seed-bearing plants in a dioecious population may be one of the selective advantages of dioecy. If a random distribution of seed plants were desirable, it seems in this case, as though a plant would be far better served by evolving a more efficient dispersal mechanism than by evolving separate sexes. It also raises the question as to whether it is more important to spread the seed-bearing plants in order to escape predation, or have localised high densities of fruit in order

to attract dispersers. Of the two study species, the dioecious *Viscum capense* has the most favourable distribution for avoiding predation, but the monoecious *Viscum rotundifolium* would probably be more attractive to dispersers. Of course, it is doubtful whether this difference between the two species is as a result of their different breeding systems, as it is far more likely to be a result of their dispersal mechanisms. It should, however, be noted that no seed or floral predators were observed in the field during the study period, so in *Viscum*, avoiding seed predation may not be of any selective significance.

Quite by accident, the results of the test for agamospermy indicate a potential selective advantage of the dioecious species. If the female plants of *Viscum capense* retain the developing seed of one season until the beginning of the following flowering season, at which point they ripen, it means that each plant has the potential to channel its resources into either flowering or fruiting at the start of each season. If, in the previous year, a large percentage of the flowers of a plant are successfully pollinated, the plant is unlikely to have sufficient resources to allow for the development and ripening of these fruits. By holding ripening back until the following season, the plant can then use most of its available resources to ripen the fruits and invest a large amount of resources in the seeds, at the expense of flowering, and produce fewer flowers that season. The production of fewer flowers in the second season may well be more than compensated for by the advantage the previous season's seeds may obtain as a result of increased resource investment. These seeds and the seedlings have to be self-sufficient, particularly in terms of water, until the hosts have been successfully invaded. Additional resource allocation to seeds could, therefore, be of selective advantage in terms of increasing the chances of seedling establishment.

The fruits of *Viscum rotundifolium* ripen directly after the flowering season, so the potential for increased resource allocation to seeds at the expense of flowering does not seem likely. Whether or not this potential advantage of *Viscum capense* is due to it being dioecious, is not clear. If it was found that this was a characteristic of dioecious species only, it could possibly be inferred that it is a selected advantage of dioecy and that the monoecious species may be unable to evolve this due to complications involved in allocations to male, as well as female, function.

Since the fruits of *Viscum capense* are available from August through to November and the fruits of *Viscum rotundifolium* from December to February at Makapansgat, all of the frugivorous birds observed at Makapansgat are likely to be in residence in the valley while fruit is available, as this

period corresponds to the breeding seasons of the birds. This could well result in only a few seeds from these populations ever being dispersed outside of the valley.

Of all the frugivorous birds observed at Makapansgat, 5 of the species were among those noted in the study of mistletoe fruit dispersal at Loskop dam (Godschalk 1986). These 5 species were the yellowfronted tinker barbet, the black collared barbet, the southern black tit and the mousebirds. Of these 5 species at Loskop dam, the tinker barbet was apparently responsible for the dispersal of between 64% and 94% of the seed of all the mistletoe species in the area (Godschalk 1986) and is listed in Roberts birds of southern Africa as feeding predominantly on mistletoe seeds (Maclean 1984). Since this bird regurgitates seeds within 20 - 60 seconds of swallowing the fruits, it is highly likely that the seeds would then be dispersed within the same tree as their female parent, or at best, in a tree extremely close by. The black collared barbet and the plum-coloured starling were observed to behave in the same way as the tinker barbet at Loskop dam, and thus, Makapansgat has at least two bird species which disperse seeds in this way. Although the plum-coloured starling is not listed as being present at Makapansgat, the redwinged starling, which does occur there may also behave in the same way.

The black tit (which occurs at Makapansgat) was observed at Loskop dam to pick a fruit, squeeze it until the skin bursts open and then place the contents onto a branch. It would then hold the seed in position with one foot and remove the fleshy layer by pecking. When it had finished pecking, the seed would remain stuck to the branch by the layer of viscin (Godschalk 1986). From observations of seeds germinating in the field at Makapansgat, it was noted that seeds occurred singly and in large clumps, which seemed to confirm the above two methods of dispersal. In either case, dispersal is likely to take place over extremely short distances, and seeds would most probably remain in the tree in which they originated.

Mousebirds were the only birds at Loskop dam observed to pass the seeds in their faeces (Godschalk 1986), and are thus the birds most likely to bring about long-distance dispersal, depending upon how much time is spent in a single tree. This form of dispersal can be potentially wasteful, since a large proportion of seeds could end up on unsuitable hosts. The behaviour of the other frugivorous species observed at Makapansgat is not known, nor is it known whether or not they actually eat mistletoe fruits. However, since the tinker barbet is responsible for such a large percentage of the distribution of mistletoe seeds in other areas, and since it subsists mainly on mistletoe fruit, it is likely that it makes a similar contribution to dispersal at Makapansgat.

Although there is enough data on the behavior of the birds to explain why the plants of both species are clumped, it is unclear from this data exactly why *Viscum rotundifolium* is so much more clumped than *Viscum capense*. It has already been shown above, however, that the clumping of *Viscum rotundifolium* plants is independent of the clumping of its' host trees. Another factor which may be responsible for greater clumping in monoecious species of mistletoe is the fact that there seems to be a preference for dispersal of seed within trees which have existing seed-bearing females. The population distribution of *Viscum capense* showed that trees which contained at least one female had, on average, higher numbers of plants per tree than those trees which had male plants only. This finding was confirmed even more convincingly by another dioecious species of mistletoe, *Viscum combreticola* Engl., in the Magaliesberg, where the average number of plants per tree was 10.72, s =8.46, for trees with females and 2.0, s = 1.0, for trees with male plants only (House 1987). This finding shows that either many seeds are dispersed within the same tree as their female parent, or that the dispersal agents move from a tree where they have just fed, to another tree with fruit bearing females where the seeds are regurgitated. The genetic data which was obtained had the potential to resolve this, but due to the genetic homogeneity of the population, was unable to do so. In either case however, it is obvious that fewer seeds are dispersed to trees which do not have fruit-producing plants. If this is a real trend, it means that monoecious species should show strong clumping tendencies compared with dioecious species. Further confirmation for this comes from the data indicating a higher mean plant-to-plant distance for the male plants of *Viscum capense* (3.2m), than was found for the between-females distance (1.34m), and the mean male-to-female distance (1.55m).

In the light of this data, it is possible to show that the degree of clumping of these species can depend on their breeding systems to a small extent. This is as a result of the presence of male plants in dioecious populations which are unattractive to dispersal agents and would therefore, not be likely to accumulate further plants in their vicinity. This would have the effect of increasing the mean plant-to-plant distance of the population. The distribution of seed-bearing plants however, remains unchanged by the presence of males in the population and is, therefore, unaffected by the breeding system of the species. Thus, if a more random distribution of seed-bearing plants were of selective advantage, dioecy would not help to increase the distance between seed-bearing plants. As mentioned earlier, if there had been selection for larger

distances between seed-bearing plants, an improved dispersal mechanism would have been of greater selective advantage.

GENETIC DATA

From data obtained in the ecological and reproductive sections, it was shown that both species had extremely limited gene flow as a result of both pollination and seed dispersal, since both pollination and dispersal are likely to take place more often within trees than between trees. It was hoped that the levels of genetic heterozygosity in the species would give some indication as to whether a dioecious breeding system had increased the levels of heterozygosity by forcing outcrossing. If this were the case, there would have been clear cut evidence for a selective advantage of dioecy.

The fact that a large number of loci in both species were fixed for heterozygosity was highly unusual, since even translocation heterozygotes are not correlated with high levels of heterozygosity (Bloom 1977, Levin 1975, Levy & Levin 1975 and Levy et al 1975). In normal sexually reproducing organisms which have more than one allele present at a locus, recombination will ensure that both homozygotes and heterozygotes will occur in the next generation. Since every plant in the populations of *Viscum capense* and *Viscum rotundifolium* was sampled, the results cannot be explained away as sampling error. Some mechanism must, therefore, be in operation to ensure high levels of heterozygosity in these species and some possible explanations will be discussed later.

For the purposes of this study however, the results are very interesting since they show no significant difference between the levels of heterozygosity between the monoecious and dioecious species of *Viscum* at Makapansgat, or elsewhere. The average percentage heterozygosity is slightly lower in *Viscum rotundifolium*, but only by 5.5%, and this is primarily due to the total lack of heterozygotes at the ME2 locus. This is also an unusual situation, but from the allele frequencies only seven heterozygotes were expected, making the assumption that random mating is occurring in the population. The data on pollination and dispersal indicates that gene flow is more likely to be highly restricted in this species, so the results for this locus could then be explained by restricted gene flow. This explanation is to some extent confirmed by the actual spatial distribution of the genotypes within the population. All but four plants in the population were fixed for the genotype BB at the ME2 locus. Only four plants had the genotype CC and

these were all located in a single tree.

The percentage heterozygosities calculated for the other populations of *Viscum capense* and *Viscum rotundifolium* were not combined with those percentages obtained for the populations at Makapansgat to determine an average level of heterozygosity for each species. This was not done since the other populations were sampled in order to confirm the presence of loci fixed for heterozygosity and results were obtained for these loci only. The loci which exhibited variability at Makapansgat were not investigated so the percentage of heterozygosity obtained for these populations is, therefore, artificially high as can be seen in the results.

One interesting genetic difference which is present between the monoecious and dioecious populations is that in the dioecious species, only one locus out of twelve is monomorphic for a single allele. In *Viscum rotundifolium*, three out of the ten loci are monomorphic. This could have been explained simply by invoking founder effects and saying that the population of *Viscum rotundifolium* may have been founded by one or very few individuals of limited genetic variability, since the data on dispersal strongly suggests that founder effects and drift may be very important. The situation does not appear to be that simple, however, since an examination of the other populations of *Viscum rotundifolium* as far afield as Nylsvley, the Magaliesberg and Grahamstown, shows that they too are monomorphic at these loci for the same alleles. This suggests that these monomorphic loci may be a species characteristic.

Since the loci analysed in this study only represent the merest fraction of the entire genome of each species, it is quite likely that additional monomorphic loci could be found in *Viscum capense* as well. If this were so, there would not be much difference between the species in terms of how many loci were monomorphic. Even if these results do reflect the actual states of the total genomes accurately, it would not mean that dioecy had caused an increase in heterozygosity of the dioecious species, since the mechanism which is maintaining these heterozygous loci is obviously a characteristic of both the dioecious and monoecious species.

If further electrophoretic studies do confirm a higher percentage of monomorphic loci in the monoecious species, then it could be hypothesised that, prior to the establishment of the mechanism which fixes heterozygosity, the dioecious species were able to prevent a large number of loci from becoming monomorphic due to their higher levels of outcrossing. The monoecious species, on the other hand, may have been prone to losing alleles due to high levels

of inbreeding and thus become more monomorphic than the dioecious species. If this were the scenario however, it would mean that the mechanism which is maintaining the heterozygous loci must have arisen independently in the already established monoecious and dioecious lines. Since heterozygous loci are a characteristic of both monoecious and dioecious lines, it is highly likely that the mechanism by which they are maintained was present in the common ancestor of these species. If this was the case, it means that dioecy must have evolved for some reason other than an increase in heterozygosity in the progeny of unisexual plants, since the monoecious plants would already have had high levels of heterozygosity. Thus in *Viscum*, the hypotheses related to selection for increased heterozygosity and selection against inbreeding depression, would fall away.

None of the loci for which data were obtained display genotype frequencies which approximate Hardy-Weinberg equilibrium. Obviously, the loci which are fixed for heterozygosity show the strongest departure, but even the other loci (PRX3, ME1 and ME2) differ from Hardy-Weinberg expectations, even when the adjustment has been made for population size. For PRX3, the percentage of heterozygous individuals in the population is very similar for both species at Makapansgat (64.4% and 63.6%), with the population of *Viscum rotundifolium* at Grahamstown having 68.4% of the individuals sampled heterozygous at this locus. At Nylsvley, the population of *Viscum rotundifolium* has 79% of its individuals heterozygous at this locus and in the Magaliesberg the small population of thirteen plants has all of its individuals heterozygous at this locus. In all the study populations there is, therefore, an excess of heterozygotes at this locus but some homozygotes still occur. There is no simple explanation for why this may be so, but controlled crosses between heterozygous individuals could be made and embryos from the resulting seed could be investigated electrophoretically. The results of this test would show whether or not normal recombination and segregation were taking place at this locus.

An excess of homozygotes is a much easier situation to explain. At the ME1 locus of *Viscum capense*, only five individuals out of fifty-nine were heterozygous. Similarly the ME2 locus of *Viscum rotundifolium* at Makapansgat and the Magaliesberg had no heterozygotes. This could be explained by limited gene flow in the population, so that plants in one area of the population cross mainly within their group, which has only one allele at that locus. The other allele may be present in another section of the population, but, because of a small genetic neighbourhood, these alleles are seldom brought together in any crosses. The highest percentage of

heterozygous individuals at either of the ME loci, was, strangely enough, found in the monoecious population of *Viscum capense* ssp *hoolei* at Grahamstown (39%), but there was still an excess of homozygotes in this population.

The importance of founder effects can be seen from the results recorded in table 3.18 for *Viscum rotundifolium*, as some populations do not include the full range of alleles which occur in the species as a whole. The importance of this effect in the dioecious species is not clear, since no novel alleles were found in the seven plants of *Viscum capense* ssp *capense* or the population of *Viscum capense* ssp *hoolei* at Grahamstown, that were not already present in the population of *Viscum capense* at Makapansgat.

This latter discovery does, however, confirm the close relationship between the subspecies of *Viscum capense* which had been doubted, since dioecy and monoecy are species-specific characteristics in the rest of the genus (Wiens & Tolken 1979). Other species of *Viscum* are genetically distinct, in terms of how few loci they share. One of the interesting findings of the isozyme work was that out of 21 loci investigated, only 2 were shared by both species of *Viscum*. Nei's (1972) genetic distance is calculated by determining the number of alleles taxa have in common and is used as a measure of relatedness and of divergence time (Grant 1988). Since the two study species have so few loci in common, they obviously have very few alleles in common. This has far-reaching implications since it shows that genetic identity between these species is very low. If this is the case, these species must have last shared a common ancestor an extremely long time ago, in order to account for their large genetic differences. One could have perhaps expected this situation due to the karyological information, which shows that the two species have different chromosome numbers. *Viscum capense* has n = 10 and *Viscum rotundifolium*, n = 14 (Wiens & Barlow 1979).

The genetic divergence between these species should not, however, influence the comparative results of this study too greatly as the species of *Viscum* are still very similar in many ways. All of the species are parasitic, have similar life forms and are bird dispersed with their flowers only differing in size. Marked genetic divergence does however appear to be a characteristic of the genus since two other species, *Viscum combreticola* and *Viscum spragueanum* Burtt Davy, also have some of their own unique loci (House unpublished data). This was discovered when the extraction buffers were being tested and several species of *Viscum* were sampled and run on a single gel. These findings could be used quite effectively in investigating phylogeny and this will

be discussed further in chapter 6.

The population genetic structures of *Viscum* species appear to be extremely complex and warrant further investigation. For the moment however, a few possible reasons for this complexity can be proposed.

From the outset, it was assumed that both species of mistletoe under investigation were reproducing sexually and the only departure from this condition known at that stage, was that some plants of *Viscum capense* ssp *capense* exhibited translocation heterozygosity at meiosis (Wiens & Barlow 1979), but would however still reproduce sexually. As a result of this, it was expected that some linkage would occur in these plants, leading to unexpected frequencies of some genotypes in these plants. Since translocation heterozygosity was reputedly not a feature of the monoecious *Viscum* species (Wiens & Barlow 1979), the results obtained in which all members of both species were fixed for heterozygosity at a number of loci, were extremely interesting.

The results could be explained away easily, if it could be shown that all the plants exhibited translocation complexes similar to those of *Oenothera* (Cleland 1972). If this were so, all the loci which were fixed for heterozygosity and those which were monomorphic for a single allele, would be located on chromosomes involved in the chromosome rings. This would effectively prevent those gene combinations from being broken up, and thus prevent further homozygotes from appearing in the population. The monomorphic loci could also be explained in this way if a null allele occurred in one of the segregation groups and a single allele was present in the other group. Alternatively these loci may not be involved in translocations and could simply be indicative of a founder effect of the whole population arising from very few founder individuals of limited genetic diversity. This situation may often be the case (Wiens & Barlow 1979), and the data collected on dispersal in this study seems to confirm this. Since the populations are small, the chance effects of drift could also account for monomorphic loci. Those loci which were found to be closer to Hardy-Weinberg expectations, would be located on chromosomes not involved in the ring complexes which would then undergo normal crossing over and segregation but would also be exposed to founder effects and drift.

Translocation heterozygosity in *Viscum* is however, not the same as the permanent translocation

heterozygosity in *Oenothera*. In the dioecious species of *Viscum*, numerous structural homozygotes are found, and in the monoecious species (apart from *Viscum capense hoolei*), no translocation rings are found (Wiens & Barlow 1979).

An alternative reason for these genetic results which would not contradict Wiens and Barlow's findings, could be that the plants are agamospermic and hardly reproducing sexually at all. This would account for the high genetic identity observed between individuals in the population and could also explain the loci which are fixed for heterozygosity. Agamospermy was tested for, but the results, as mentioned, were unfortunately inconclusive. There is, however, another reason for doubting the presence of large scale agamospermy, at least in *Viscum capense*. If sex were genetically determined in this species, as proposed by Wiens and Barlow (1979), then large scale agamospermy would result in a highly skewed sex ratio in favour of females, as males could only be produced by sexual reproduction. Since the sex ratio is effectively 1:1, it is unlikely that agamospermy is occurring at high enough levels to produce the genetic structure of this population of *Viscum capense*.

Since a sex ratio is obviously unobtainable for the monoecious *Viscum rotundifolium*, agamospermy cannot be ruled out for this species, but since it exhibits the same population genetic structure as *Viscum capense*, it seems more likely that the same mechanism is responsible for genetic structure of both species.

The literature available on isozyme studies of other species which exhibit translocations, was investigated in order to compare the genetic structure of these populations with findings in other studies. Although it is generally assumed that translocation heterozygosity could maintain higher levels of heterozygosity under conditions of inbreeding than could be maintained by structural homozygotes, studies using electrophoresis of isozymes have indicated that this is not necessarily so. Levin (1975), using studies in which results were obtained for at least 7 enzyme loci, shows that no clear relationship exists between structural heterozygosity and genic heterozygosity. Other studies show low levels of heterozygosity and variability as well as few polymorphic loci and generally only 2 alleles present at each locus (Bloom 1977, Levy & Levin 1975 and Levy et al 1975). None of these studies reported loci fixed for genetic heterozygosity in any of the species studied which had translocation complexes. The results obtained for the two species used in this study, cannot therefore, be explained by translocation heterozygosity, and if reproduction is taking place sexually, some other cytological mechanism must be at work

to produce this situation.

Both the monoecious and the dioecious species of *Viscum* in this study exhibit similar population genetic structures in terms of having some loci fixed for heterozygosity, some monomorphic and a few loci at which recombination appears to be taking place. Thus it seems likely that the same cytological mechanism must be responsible for the genetics in both species, particularly since the same enzymes which are heterozygous in one species behave the same way in the other species, even though they are not the same loci in both species. Similarly, the enzyme coding loci which do exhibit recombination are also the same in both species. If the same cytological mechanism is operating in both species, translocation heterozygosity cannot be the mechanism since it does not occur in *Viscum rotundifolium*.

Another explanation for the loci with fixed heterozygosity could be that the species of *Viscum* are ancient polyploids, which have since become diploidised, resulting in more than one copy of each locus. This would result in higher levels of heterozygosity, since there would be more than one copy of each locus, increasing the possibility of two different alleles being present. This theory is based on work which shows that allopolyploids have increased numbers of isozymes due to the addition of divergent genomes (Crawford 1985, Gottlieb 1981,1982 and Soltis & Soltis 1988). Some homozygotes should, however, still occur. The base chromosome number for the genus is n = 14, so an ancestor with n = 7 is possible. *Viscum capense* has n = 10, and even in this case the ancestral condition could have been n = 5. Electrophoretic data can be used to test for the presence of polyploidy, since the number of isozymes is highly conserved in diploid seed plants (Gottlieb 1981 and 1982). The two study species of *Viscum* do not show an increase in isozyme number over what one would expect in a diploid plant, so there is no direct evidence for polyploidy from this. Polyploidy can escape this type of electrophoretic detection, however, if allopolyploidy was followed by massive gene silencing, or if autopolyploidy had occurred resulting in double doses of genes but no new isozymes from different lineages, as in the case of allopolyploidy (Soltis & Soltis 1988). Polyploidy and subsequent episodes of massive gene silencing, could also explain the large genetic divergence between the species of *Viscum* since different loci could have been silenced in different species.

Heterozygosity can also be fixed in a species, if two alleles are incorporated into the genome as two separate gene loci as a result of unequal crossing over between two homologous chromosomes during meiosis (Ohno 1970). It seems unlikely, however, that these types of

duplications could have occurred to the extent required to explain the large number of loci which were heterozygous in these species.

A further possibility is that there could be severe selection against homozygotes at these loci, so that adult populations consist of heterozygotes only. In the genus *Oenothera*, there are lethal genes which prevent structural homozygotes from developing (Cleland 1972). This cannot be occurring in *Viscum*, however, since translocation heterozygosity is not a feature of all the dioecious plants and it does not occur in the monoecious species (Wiens & Barlow 1979).

Although the data collected in this study do not allow one to explain the genetic mechanisms which result in the levels of heterozygosity observed, this was not the object of the study. The data do, however, fulful one of the aims of the study, which was to provide an answer to the question as to whether the dioecious species has a higher level of genetic heterozygosity than the monoecious species. Since there is no difference in the levels of heterozygosity of both species, it seems likely that selection for outcrossing did not lead to the evolution of dioecy in *Viscum*.

The survey of the levels of heterozygosity in the study populations seems to show that each meiotic event in these species of *Viscum*, is able to include features of both sexual and asexual reproduction, since some loci exhibit segregation and others do not. This ability is similar to that of the polyploid *Rosa canina*, although in *Rosa* the mechanism by which this is maintained is unequal synapsis (Fagerlind 1944, Gustafsson 1944 and Gustafsson & Hakansson 1942, in Richards 1986). The ability to to do this, potentially has some great advantages, since all of the genes which result in high levels of fitness could be transferred to the chromosomes or linkage groups, which are inherited without undergoing recombination, and in this way favourable gene combinations could be preserved (Richards 1986). Similarly, deleterious genes could be transferred to the chromosomal regions which do recombine and thus they could be exposed to selection and thus eliminated from the gene pool (Richards 1986). An extensive cytological survey of *Viscum* species would have to be undertaken in order to explain the maintenance of this system in this genus. Since Wiens and Barlow's survey (1979) shows that translocation complexes do not occur in *Viscum rotundifolium* and some plants of *Viscum capense* ssp *capense*, they cannot be used to explain the above situation. Any future cytological study should also attempt to confirm Wiens and Barlow's (1979) findings.

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Name of thesis The Consequences Of Monoecy And Dioecy In Congeneric Species, And Their Implications For The Evolution Of Dioecy In The Genus Viscum. 1989

PUBLISHER:
University of the Witwatersrand, Johannesburg
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