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Translocation heterozygosity in southern African species of *Viscum*

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ABSTRACT

Sex-associated and floating translocation complexes are characteristic of dioecious species of *Viscum*, but are virtually absent in monoecious species. The majority of dioecious species has fixed sex-associated translocation complexes with the male being the heterozygous sex. The sex-associated multivalent is usually ○4 (ring-of-four) or ○6, rarely ○8. Dioecious species without sex-associated translocations are much less common. Most of the dioecious species are also polymorphic for floating translocations, producing one or more additional multivalents ranging from ○4 to ○12. Floating translocations may be more frequent in species that do not have sex-associated translocations. Supernumerary chromosomes are also present in several species. Sex ratios are at unity in most dioecious species, but female-biased ratios may occur in some species. The high correlation between dioecy and translocation heterozygosity suggests that translocations are primarily associated with the origin and establishment of dioecy. Any rôle in the maintenance of biased sex ratios through meiotic drive is probably secondary. Sex-associated translocations may serve to stabilize dioecy by bringing the sex factors into close linkage. Subsequent structural rearrangements within a sex-associated translocation complex may bring the sex factors together in one chromosome pair, releasing floating translocations. The high frequencies of floating translocation heterozygosity in some species indicate that such heterozygosity also has adaptive value.

RÉSUMÉ

TRANSLOCATION HÉTÉROZYGOTE CHEZ DES ESPÈCES SUD-AFRICAINES DE VISCUM

Des complexes de translocation liés au sexe et flottants sont caractéristiques des espèces dioïques chez Viscum, *mais n'existent pratiquement pas dans les espèces monoïques. La plupart des espèces dioïques ont des complexes de translocation fixes et liés au sexe, avec l'hétérozygotie du côté mâle. Le multivalent associé au sexe est habituellement ○4 (anneau de quatre) ou ○6, rarement ○8. Les espèces dioïques sans translocations liées au sexe sont beaucoup moins répandues. La plupart des espèces dioïques sont également polymorphiques pour des translocations flottantes, produisant un ou plusieurs multivalents additionnels qui s'étendent de ○4 à ○12. Des translocations flottantes peuvent se rencontrer plus fréquemment chez des espèces qui n'ont pas de translocation liée au sexe. Des chromosomes surnuméraires se rencontrent également chez plusieurs espèces. Dans la plupart des espèces dioïques la sex-ratio est de 1, mais dans certaines on peut trouver une déviation du côté femelle. Le degré élevé de corrélation entre la dioécie et l'hétérozygotie de la translocation suggère que les translocations sont associées primairement à l'origine et a l'établissement de la dioécie. Tout rôle que pourrait jouer la poussée méiotique dans le maintien de sex-ratios faussées est probablement secondaire. Des translocations liées au sexe peuvent servir à stabiliser la dioécie en aboutissant à une association étroite entre les facteurs sexuels. Des réarrangements structurels ultérieurs dans l'enceinte d'un complexe de translocation lié au sexe peuvent rassembler les facteurs sexuels dans une seule paire chromosomique en relâchant des translocations flottantes. La fréquence élevée des translocations hétérozygotes flottantes chez certaines espèces indique que pareille hétérozygotie possède également une valeur adaptative.*

Viscum is a genus of mistletoes comprising about 100 species with an extensive range in the Old World. Its species parasitize a wide range of dicotyledonous trees and shrubs in a variety of forest and woodland habitats. Major centres of species diversity are Africa and Madagascar, but there is also a significant development of the genus in tropical and subtropical Asia. The extremes of its geographical range are reached in Europe, southern Africa, temperate Asia, and Australia. In southern Africa the genus is represented by at least 17 species, of which 9 are dioecious, 7 monoecious and one (*V. capense*) that has both monoecious and dioecious subspecies.

The flowers in *Viscum* are small and consistently unisexual. The basic inflorescence unit is a cymule, usually consisting of three minute flowers (1–3 mm wide), but in some species the flowers are solitary or in larger clusters. The monoecious species typically have the central flower of the cymule of one sex and the two lateral flowers of the other.

Dioecious species are common in Africa and Madagascar, and a few others are widely distributed in Europe and temperate Asia, but monoecious species are the rule in tropical Asia. In the dioecious species the cymules are still typically three-flowered, so that dioecy presumably involves a possibly simple conversion of all flowers to the same sex for all cymules of a plant. Dioecy appears to be highly stable in its expression in *Viscum*, and mixed cymules are unknown in any of the dioecious entities we have studied.

The most common chromosome number among the African species of *Viscum* is $x=14$, and this is apparently the basic number for the genus (Wiens, 1975). The Madagascan species so far examined mostly have $x=13$, but more extensive data are needed before firm conclusions can be drawn. Some of the Madagascan species are more closely related to the species of *Viscum* of the Asian region than to those of Africa. The southern African species are especially interesting because of the high incidence of derived aneuploid chromosome numbers of $x=15$, 12, 11 and 10 occurring in this region.

Translocation heterozygosity was first reported for *Viscum* in the dioecious species, *V. fischeri* Engl. from eastern Africa (Wiens & Barlow, 1973; 1975). This species has a unique system of translocation heterozygosity in which male plants have a chromosome number of $2n=23$, and consistently produce seven bivalents and an open multivalent chain of nine chromosomes at meiosis. Female plants have a chromosome number of $2n=22$, form 11 bivalents at meiosis, and are thus chromosomally homozygous for the 11-chromosome genome. Translocation heterozygosity is therefore presumed to be sex-associated, with the male being the heterozygous sex and the 11- and 12-chromosome genomes female-determining and male-determining, respectively (Barlow & Wiens, 1976). Additional studies

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also revealed the presence of a strongly female biased sex ratio of approximately 1: 2.

Further studies have indicated that sex-associated translocations are widespread in dioecious species of *Viscum*, but typically absent in monoecious species. A more extensive cytogenetic survey of the southern African species of *Viscum* is presented in this paper. The occurrence and distribution of translocaton heterozygosity and supernumerary chromosomes are described and considered in relation to the origin and establishment of dioecy and to variations in the sex ratio.

METHODS

Flower buds were fixed in ethyl alcohol/acetic acid (3:1) and stored in absolute alcohol at −15 °C whenever possible. Chromosomal constitution was determined from aceto carmine squash preparations of pollen mother cell, or embryo sac mother cell meiosis in some female plants. The latter involved dissection of a block of tissue containing the sporogenous cells from the base of the flower bud (there are no discrete ovules in *Viscum*), maceration in a solution of 1% HCl in 45% acetic acid on a slide for 2 min at 60° C, and light squashing.

The sex ratio for most species was determined directly from established populations in the field. Plants were mostly removed from the host for counting so that difficulties caused by clumped distributions and autoparasitism could be minimized.

Voucher specimens for each chromosome determination are deposited at the State Herbarium of South Australia (AD), Botanical Research Institute, Pretoria (PRE), and the Garret Herbarium, University of Utah (UT).

RESULTS

The chromosomal constitutions of 359 individuals were determined for 16 of the 17 species of *Viscum* currently recognized for southern Africa (Wiens & Tölken, 1979). Details of these determinations are given in the Appendix and selected characteristic configurations are illustrated in Fig. 1.

Monoecious species

All of the monoecious species were studied karyologically with the exception of *V. schaeferi*. In all but one of these the floral distribution is essentially constant and generally typical of monoecious species, with each cymule bearing both male and female flowers, with a central flower of one sex and lateral flowers of the other. In *V. capense* subsp. *hoolei*, however, the expression of monoecy is altered. Some plants produce mostly female flowers which possess basically female morphology, while other plants produce mostly flowers of typical male morphology and mature few fruits. The vegetative characters of *V. capense* subsp. *hoolei* agree generally with the dioecious *V. capense* subsp. *capense*; thus in subsp. *hoolei* monoecy probably represents a derived state resulting from modification of sex determination in a dioecious species. In other monoecious species of *Viscum*, e.g. *V. obovatum*, male and female flowers are produced together only at certain times, while at other times only female flowers occur in the bracteal cups (Wiens & Tölken, 1979). This is apparently a relatively common occurrence in monoecious species in other parts of the world (Danser, 1941).

The chromosomal data for the monoecious entities are summarized in Table 1. There is a general absence

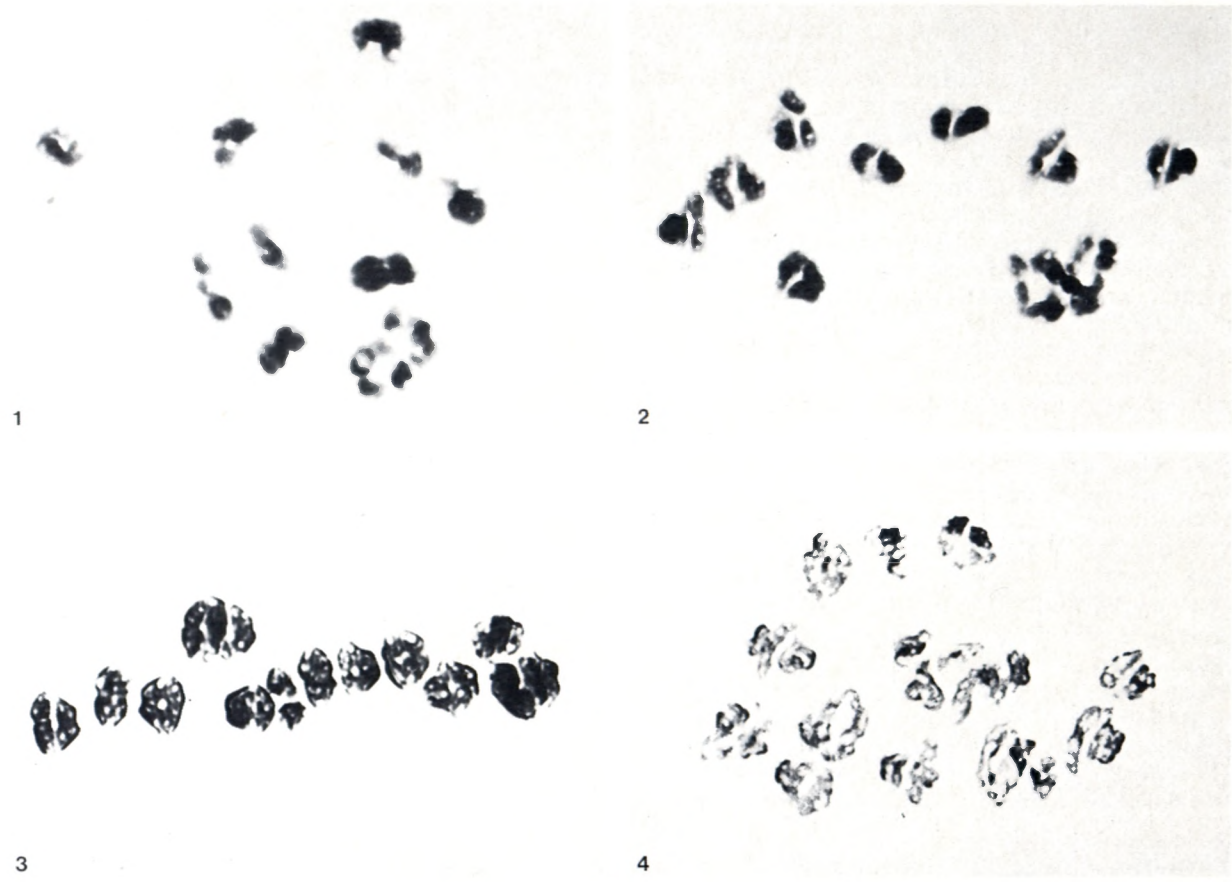


FIG. 1.—Pairing configurations of metaphase I chromosomes in African species of *Viscum*. 1, *V. subserratum* 9II ○4 (W5305h). 2, *V. subserratum* 8II ○6 (W5305f). 3, *V. continuum* 10II ○4 (W5377b). 4, *V. obscurum* 10II ○4 ○6 (W5372b).

of translocation heterozygosity in these species, its only occurrence being recorded in a single individual of *V. capense* subsp. *hoolei* which has chromosome associations of eight bivalents and a ring of four chromosomes (8II+○4). This is in striking contrast to the situation in the dioecious species where translocation heterozygosity is probably universal, at least in some male plants (see below). As previously mentioned, in *V. capense* subsp. *hoolei* monoecy may be derived from dioecy, so that the translocation heterozygosity present may be the same as that found in the dioecious subspecies.

TABLE 1.—Summary of chromosome constitutions in southern African monoecious species of *Viscum*

	Chromosome no. (n)	No. of plants (Bivalents only)	No. of plants (Floating ○4)
V. capense subsp. *hoolei**	10	10	1
V. minimum	14	1	
V. nervosum	14	6	
V. obovatum	12	5	
V. rotundifolium	14	20	
V. spragueanum	23	1	

* atypical monoecy: see text.

Chromosome numbers among the monoecious species are $n=10$, 12, 14 and 23, but $n=28$ occurs in some monecious species in eastern Africa (Wiens, 1975). *Viscum spragueanum* is probably consistently polyploid, but one tetraploid individual was discovered in *V. capense* subsp. *hoolei*, which is otherwise diploid. This situation contrasts sharply with that in the dioecious species, where only a single triploid plant was discovered in *V. verrucosum*. Although alternative explanations are possible, the rarity of polyploidy in the dioecious species could be related to a mechanism where sex is determined by a balance between strongly female- and male-determining X and Y factors, as originally proposed by Muller (1925).

Dioecious species

The chromosome constitutions of all of the dioecious southern African species of *Viscum* were analysed (Table 2). Translocation heterozygosity occurs in all 10 of the dioecious species, and these fall into two groups. The larger group (with 6 species) exhibits patterns in which the male plants are all heterozygous for at least one translocation complex. Only a few female plants were analysed for pairing relationships in the southern African species, but these were consistent with the assumption that *some* translocation rings are sex associated, occurring in male plants only, and that female plants are predominantly chromosomally homozygous and produce mostly bivalent associations. Thus where male plants occur with more than one translocation ring, the additional rings presumably also occur in female plants. The sample sizes in the southern African species are not large enough to predict the frequencies with which these floating translocations occur in female plants, but there is no reason to assume they are not identical to those in the male plants, as previously demonstrated in an east African species, *V. hildebrandtii* (Wiens & Barlow, 1979). In that species the population from Riandu, Kenya, has male plants which all possess ○6 (the sex-associated complex), but some male plants have an additional ○4 and/or ○6. In the female plants these floating ○4 and ○6 were recorded as well as a homozygote with 14 II. This strongly supports our earlier suggestion (Barlow & Wiens, 1975) that in this group of dioecious *Viscum* there is one translocation ring which is sex-associated and fixed in males as the heterozygous sex, while the other rings are floating and not sex-associated.

In some respects the species in this group are chromosomally relatively uniform. With the exception of *V. subserratum* ($n=11$), all species in the group have $n=14$ and a generally symmetrical karyotype. The most common sex-associated rings are ○4 and ○6, and in three species both rings are present. A floating ○4 is present in at least five of the six species.

TABLE 2.—Summary of choromosome constitutions in dioecious species of *Viscum* in southern Africa

	n=	No. and pairing relationships of male plants												No. and pairing relationship of female plants			Fixed rings		Floating rings		
		bivalents only	○4	○6	○4 ○4	○8	○4 ○6	○10	○4 ○8	○12	○4 ○4 ○6	○4 ○6 ○6	○4 ○4 ○8	bivalents only	○4	○4 ○6	○4	○6	○8	○4	○6
Species with fixed rings in male plants:																					
V. anceps	14		8	2	3		1										×	×		×	
V. combreticola	14			3		1					1		1					×		××	
V. continuum	14		54	1	5												×	×		×	
V. menyharthii	14		2	7	1		4				2	1		1	2		×	×		×	×
V. subserratum	11		19	1	9									2	1		×	×		×	
V. verrucosum	14		48	8	20		1										×	×		×	
Species lacking fixed rings in male plants:																					
V. capense.	10	14	7												1					×	
V. crassulae	12	28	7	1																×	×
V. obscurum	15	10	11	1	4		5	2	2	1						1			×	×××	×
V. oreophilum	14	3		1	5															××	×

A few differences are superimposed on this common pattern. A sex-associated ○8 occurs in *V. combreticola*, and in two additional species in eastern Africa (Wiens & Barlow, 1979). Its occurrence may be linked with the presence of a sex-associated ○6, which appears to be common in these three species. In *V. combreticola* there is also evidence for two independent floating ○4, and a floating ○6 occurs in *V. menyharthii*.

In this species group, then, the sex-associated complex consistently appears in males as ○4 and ○6, and in addition, most species have a floating ○4. This regular pattern suggests that the sex-associated translocations (and possibly also the floating ones) may be the *same* in most or all of the species in the group. If so, the translocation system may be relatively old and possibly established prior to the differentiation of the extant species. The accumulation of linked complexes through the translocations may have promoted rapid differentiation of biotypes, and the uniformity of chromosome number among these species is consistent with this possibility.

The second group includes the remaining four species which all have different chromosome numbers ($n=10$, 12, 14, 15). In these species some of the male plants produced only bivalents, indicating that fixed sex-associated complexes are not present. There is evidence, however, of extensive floating translocation heterozygosity, presumably in female plants as well as males, but additonal data on this point are needed. In fact, floating translocations are apparently more frequent in this species group than in the first. In *V. oreophilum* two floating ○4s occur, but *V. obscurum* contains the most extreme complexity, where associations up to ○12 exist, and individuals with two rings are relatively common. The minimum number of floating translocations needed to produce these associations in *V. obscurum* is five, assuming that all configurations observed represent recombinations of the same translocations.

Perhaps the species in this group represent a transitional stage in the evolution of the sex-associated translocation system, with the floating translocations acquired first and later linked with the sex-determining chromosomes by subsequent translocations. However, this group contains both highly specialized and diverse species as indicated by their morphology and range of chromosome numbers. They may, therefore, also represent a derived state in which sex-associated multivalents are released from linkage with the sex-determining chromosomes by subsequent translocations. These would then become floating multivalents. This hypothesis is consistent with the observation that the species in this group have a greater diversity of floating multivalents than the species in the preceding group. In other words, sex-associated multivalents were possibly acquired early in the evolution of the genetic system, and later converted to floating associations by subsequent exchanges. This appears to us as the best explanation and is discussed further.

A third class of translocation heterozygosity occurs in African *Visca*, but it is limited to the single species, *V. fischeri*, which is geographically restricted to eastern Africa. This translocation system was described earlier in this paper and extensive discussion of this translocation system is unnecessary and it is mentioned here only for conceptual completeness. This species is unique in having different chromosome numbers in males and females and also unusual in its uniformity, having the same sex-associated multivalent in all male plants examined, and no floating multivalents in either sex.

Frequencies of floating translocations

The data indicate that the frequencies of floating translocations vary between populations in some species. Estimates of the frequencies of floating ○4 for a few species in which some analysis is possible are given in Table 3.

In *V. verrucosum* the frequency of the heterozygote with the floating ○4 may vary significantly between populations, from zero to the theoretical maximum of 0,5, with corresponding variations in gametic frequencies of the translocation complexes. Similar variations appear to occur in some of the other species listed. However, because of the small sample sizes it is not possible to determine the frequencies

TABLE 3.—Frequencies of floating ○4 in southern African species of *Viscum*

Species	Locality (for details see Appendix)	No. of plants sampled	No. of plants with floating ○4	Frequency of heterozygotes	Estimated gametic frequency of translocation	Heterogeneity χ^2	Heterogeneity P
V. capense capense	Cape Peninsula	14	8	0.57	0.50*	8.69	<0.005**
V. capense hoolei	Grahamstown	8	1	0.13	0.07	f=1	
V. crassulae	King William's Town	8	0	0.00	0.00	5.35	<0.025**
	29–37 km N of Grahamstown	15	7	0.47	0.37	f=1	
V. subserratum	Ntokweni	8	2	0.25	0.15		
	Duiwelskloof	8	2	0.25	0.15	1.27	0.5
	Mkuze	11	5	0.45	0.35	f=1	
V. verrucosum	Duiwelskloof	8	5	0.63	0.50*		
	Soutpan	16	1	0.06	0.03		
	Mkuze	12	0	0.00	0.00	18.9	<0.005**
	Kranzkop	14	3	0.21	0.12	f=4	
	Siteki	13	7	0.54	0.50*		

* theoretical maximum.
** significant at $p<0.025$.

TABLE 4.—Sex ratios in some dioecious southern African species of *Viscum*

Species	Locality (for details see Appendix)	No. of plants		Sex ratio	χ^2 exp. 1.00	χ^2 exp. 0.75
		Male	Female			
V. anceps	12 km W of Port St Johns	12	11	1.09	0.04	
V. capense	Cape Peninsula	133	110	1.21	2.18	
V. continuum	Calitzdorp	9	11	0.82	0.20	
	De Rust	28	33	0.85	0.41	
	Oudtshoorn	59	60	0.98	0.00	
	Laingsburg	54	47	1.15	0.49	
	Worcester	16	19	0.84	0.26	
	Bonnievale	29	48	0.60	4.69*	
	Stormsvlei	13	14	0.93	0.02	
		208	232	0.90	1.30	
V. crassulae	King William's Town	10	6	1.67	0.50	
V. menyharthii	Wyliespoort	22	19	1.16	0.22	
V. obscurum	Pretoriuskop	5	6	0.83	0.09	0.03
	King William's Town	29	28	1.04	0.02	1.52
	Grahamstown	44	70	0.63	5.93*	0.86
	Avontuur	39	48	0.81	0.93	0.16
		117	152	0.77	4.55*	0.05
V. subserratum	Ntokweni	36	49	0.73	1.99	0.01
	Mkuze	31	42	0.74	1.66	0.00
		67	91	0.74	3.65	0.01
V. verrucosum	Duiwelskloof	63	58	1.09	0.21	
	Soutpan	16	24	0.67	1.60	
	Mkuze	39	52	0.75	1.86	
	Kranzkop	52	44	1.18	0.67	
		170	178	0.96	0.18	

* $p < 0.05$.

with high levels to confidence, and the calculated frequencies in Table 4 can only be regarded as a general indication of the scale of variation.

If the high frequencies of heterozygosity recorded for some populations are real, then positive selection favouring the heterozygotes is indicated. If the translocation complexes maintain adaptive gene combinations, there must be interaction between suites of genes in the complementary chromosome complexes, thus generating heterozygote advantage. Bloom (1977) has shown in *Clarkia* that translocation heterozygosity is maintained because of the levels of inbreeding which might be imposed on natural populations. This argument could apply to *Viscum*, which is a generally host-specific parasite, often occurring in small, relatively isolated local populations probably derived from a few founder individuals.

Supernumerary chromosomes

A single supernumerary (B-) chromosome was observed in some individuals in a few of the dioecious species. In *V. verrucosum*, the B-chromosome was recorded from several populations throughout the range of the species in South Africa, and occurred in approximately half the plants sampled from each population in which it was found. The B-chromosome in this species was visible at meiotic metaphase as a small rounded unit usually just off the equatorial plate. Earlier in prophase it showed a constriction near one end, indicating it was acrocentric. The B-chromosome usually moved undivided to one pole at first anaphase and divided at second anaphase, and was thus transmitted to two of the four pollen grains. Occasionally it lagged at first anaphase, and the elimination of the B-chromosome may be correlated with the occurrence of a small microcyte present in less than 10 per cent of tetrads. In some cases the B-chromosome in *V. verrucosum* appeared to be associated with aberrations in the A-chromosomes. Sometimes one bivalent did not orient at first metaphase, behaving as if it were monocentric. In other cases chromosome fragmentation appeared to occur in cells carrying the B-chromosome.

In *V. capense* from the Springbok area the B-chromosome was of similar shape and behaviour to that in *V. verrucosum*, except it was larger. In *V. obscurum* a B-chromosome was observed in only one population, but it was much larger than those observed in any other species. Thus different species appear to have distinctive B-chromosomes. In addition, at least three different B-chromosomes are widespread over the range of *V. album* in Europe and Asia (Barlow *et al.*, 1978; Barlow & Wiens, unpublished data), suggesting they have a regular behaviour which ensures their persistence.

Although B-chromosomes in *Viscum* may be confined to the dioecious species, it is unlikely that they have a direct association with the dioecious state. Since the dioecious species are also characterized by high frequencies of translocations, it seems likely that the B-chromosomes have originated as centric fragments resulting from chromosome breakage, reunion and segregation. Such an origin has been suggested for other groups of plants (John, 1976; Darlington, 1974; Jackson, 1960) and particularly for *Clarkia*, where B-chromosomes may arise *de novo* rather frequently in populations with high levels of translocation heterozygosity (Wedberg *et al.*, 1968).

Sex ratio

The data on sex ratio are summarized for several species in Table 4. Sample sizes are occasionally relatively small, due to the difficulty of collecting some species. A few species of *Viscum* have deviations from a sex ratio of unity. An excess of female plants was previously recorded for *V. fischeri* in east Africa (sex ratio 0.52; Barlow & Wiens, 1976). In *V. obscurum* the total for all samples deviates significantly from an expectation of unity, and the individual samples are homogeneous and consistent with a female predominant sex ratio of about 0.75. A similar excess of female plants may occur in *V. subserratum*, although the populations sampled gave results just within the level of significance. The only case of a possible excess of male plants is in the east African species, *V. hildebrandtii* where the sex ratio may be about 1.4 (Barlow & Wiens, 1975).

In most cases, however, the data agree with a sex ratio of 1.0, and where more than one population of a species was sampled, the data appear to be homogeneous. The biased sex ratio first observed in *V. fischeri* therefore does not appear to be a general feature of the African species. No other species examined from Africa shows the distortion to the same degree, either in favour of male or female plants.

In *V. fischeri* we originally suggested that the biased sex ratio might be due to the sex-associated translocation heterozygosity, with the two translocation complexes having different transmission rates through the pollen. We believed this might be the principal rôle of the translocation heterozygosity in this species. The patterns obtained in southern Africa now indicate that this explanation is unlikely, since there is no regular relationship between sex ratio and translocation heterozygosity. If the translocations have a rôle in the maintenance of biased sex ratios, it is probably a secondary function of the translocation system and is established only in those species in which distortion of the sex ratio may be of significance for other reasons. Situations in which biased sex ratios might be favoured in plants are described by Bawa & Opler (1977), Mulcahy (1967) and Kaplan (1972). In *Viscum* an excess of females might achieve maximum seed set in populations which are space-limited, efficiently pollinated, and perhaps subject to heavy seed predation. An excess of male plants may be favoured in open situations where pollination efficiency might be limited by the distance between male and female plants.

DISCUSSION

This survey shows that translocation heterozygosity occurs in all the dioecious species of *Viscum* in southern Africa, but is apparently rare among the monoecious species. Since the dioecious species *V. album* L. and *V. cruciatum* Boiss. in Europe and Asia, and *V. alniformosanae* Hayata in Taiwan show patterns of translocation heterozygosity similar to the African species (Barlow *et al.*, 1978; unpublished data), this relationship probably extends to all dioecious members of the genus. The only remaining area rich in dioecious species of *Viscum*, which is not well surveyed, is Madagascar. The Madagascan species, as opposed to the African ones, are apparently based predominantly on $x=13$ instead of $x=14$ (Wiens, 1975), but there is little reason to suspect that their genetic systems are different.

The translocation heterozygosity in *Viscum* thus emerges as a phenomenon which is characteristic of the dioecious state. As suggested above, some of the translocations may be common to several species, and the translocation systems were possibly established prior to, or during the differentiation of related species. The translocations, in fact, may have played a fundamental rôle in the establishment of dioecy in the genus.

There is little doubt that dioecy in *Viscum* is derived from monoecy. Unisexual flowers are characteristic of all Viscaceae, and monoecy is fixed or predominant in all genera. In most other respects, however, *Viscum* conforms with the observations or predictions of dioecy in tropical forests made by Bawa & Opler (1975). They concluded that dioecy evolved in response to selection for the enforcement of outcrossing, and suggested a number of factors which may have favoured dioecy as the outcrossing mechanism. These include (1) the likely genetic simplicity of dioecy compared with self-incompatability (Baker, 1967), (2) high levels of reproductive failure in self-incompatible hermaphrodite or monoecious species because of the small foraging ranges of the pollen vectors, and (3) escape from seed predation caused by altered size and distribution of seed set. Possibly all of these factors have contributed to the evolution of dioecy in *Viscum*.

If dioecy is established by mutations affecting different hormone systems which favour maleness and femaleness (Audus, 1972; Bose & Nitsch, 1970), then such genes would likely be nonallelic. This possibility was argued on *a priori* grounds for angiosperms generally by Ross & Weir (1976) and Charlesworth & Charlesworth (1979). These authors also point out that mutations for dioecy are not likely to accumulate simultaneously, and that in many cases gynodioecy, through male sterility, is an intermediate step. If the genes for full dioecy were not linked, recombination could produce males, females, hermaphrodites and neuters, and Charlesworth & Charlesworth (1979) suggest there might often be a "linkage constraint', such that unlinked genes for dioecy may be selected against. Thus genes for dioecy should occur on the same chromosome, with no crossing-over between loci, so that particular chromosomes become identified with sex determination. If these constraints apply in *Viscum*, then translocations should have a rôle in the evolution of dioecy, namely in bringing the nonallelic sex factors into close linkage. Our data suggest that in many species of *Viscum* dioecy is stabilized in this way, and that this system for maintaining dioecy is conserved during the differentiation of new species. The problem is discussed more fully elsewhere (Wiens & Barlow, 1979).

Even if this model for the evolution of dioecy in *Viscum* is correct, translocations are perhaps of further adaptive importance in the genus. Floating translocations are common even in species like *V. hildebrandtii* which retain the sex-associated multivalent, and they are also retained in species like *V. obscurum* which may have lost the sex-associated multivalent. Their persistence in high frequency indicates a more basic rôle in the genetic system of *Viscum*, presumably as a means of maintaining high levels of heterozygosity. But why have translocations accumulated only in dioecious species and not in monoecious species? Perhaps the fixation of translocation heterozygosity by sex-association allows time

Chromosome constitution in species of Viscum in southern Africa

Species	Monoecious or Dioecious	Gametic chromosome No.	Locality	No. and sex of plants	Configuration	Voucher or reference
V. anceps E.Mey. ex Sprague	D	14	SOUTH AFRICA: Natal: Oribi Gorge	1 m	1111106	W5358b
				1 m	9II0406	W5358a
			TRANSKEI: 27-38 km N of Port St. John	4 m	12II04	W5362a,c W5363 W5364b
				1 m	1111106	W5362b
				2 m	10II0404	W5364a W5365
			TRANSKEI: 12 km W of Port St. John	3 m	12II04	W5366b,d,g
				1 m	10II0404	W5366a
			TRANSKEI: 23 km W of Butterworth	1 m	12II04	W5368
V. capense L.f. subsp. capense	D	10	SOUTH AFRICA: Cape: Springbok	6 m	10II[1]	W5413 W5414 W5416a-d
			SOUTH AFRICA: Cape: Garies	2 m	10II	W5412a,b
			SOUTH AFRICA: Cape: Cape Peninsula, from Chapman's Bay to Nature Res.	6 m	10II	B2178a-c B2182d,e,i
				7 m	8II04	B2179a B2182a-c,f-h
				1 f	8II04	B2178d
V. capense L.f. supsp. hoolef Wiens	M[2]	10	SOUTH AFRICA: Cape: 7 km S of Prince Albert	1	tetraploid	W5402
			SOUTH AFRICA: Cape: 17 km S of Willowmore	3	10II	W5393 W5394a,b
			SOUTH AFRICA: Cape: 8 km NW of Grahamstown	7	10II	W5385a,b,d-h
				1	8II04	W5385c
V. combreticola Engl.	D	14	SOUTH AFRICA: Transvaal: 18 km E of Waterpoort	2 m	1111106	W5316a,c
			SOUTH AFRICA: Transvaal: 9 km W of Zoutpansberg summit	1 m	1111106	W5313b
			SOUTH AFRICA: Transvaal: N of Middelburg near Tigerpoort	1 m	10II08	W5271e
				1 m	6II040408	W5271a
			SOUTH AFRICA: Transvaal: Hartebeestpoort	1 m	7II040406	W4672
V. continuum E.Mey. ex Sprague	D	14	SOUTH AFRICA: Cape: 26 km W of Komga	3 m	12II04	W5371a-c
			SOUTH AFRICA: Cape: 9 km E of Fort Beaufort	5 m	12II04	W5377a,c-e,g
				2 m	10II0404	W5377b,f
			SOUTH AFRICA: Cape: 4 km E of Calitzdorp	6 m	12II04	W5397a-f
			SOUTH AFRICA: Cape: De Rust	5 m	12II04	W5398a-c,e,f
				1 m	1111106	W5398d
			SOUTH AFRICA: Cape: 4 km N of Oudtshoorn	6 m	12II04	W5400a,b,d-g
				1 m	10II0404	W5400c
			SOUTH AFRICA: Cape: 7 km S of Prince Albert	3 m	12II04	W5401a-c
				2 m	10II0404	W5401d,e
			SOUTH AFRICA: Cape: 68 km E of Laingsburg	6 m	12II04	W5403a-f
			SOUTH AFRICA: Cape: 16 km S of Worcester	8 m	12II04	W5405a-h
			SOUTH AFRICA: Cape: 20 km S of Bonnievale	6 m	12II04	W5406a-f
			SOUTH AFRICA: Cape: 13 km N of Stormsvlei	6 m	12II04	W5408a-f
V. crassulae Eckl. & Zeyh.	D	12	SOUTH AFRICA: Cape: 74 km SW of King Williams Town	8 m	12II	W5373a-e,g,i,j
			SOUTH AFRICA: Cape: Committee Drift between Grahamstown and Fort Beaufort	4 m	12II	W5376a-c,f
			SOUTH AFRICA: Cape: Ecca Pass NE of Grahamstown	4 m	12II	W5378a,e-g
			SOUTH AFRICA: 29-37 km N of Grahamstown	7 m	12II	W5379b,c,f,h, W5381a,b,d
				7 m	10II04	W5379a,d,e,g,i,j W5381c
				1 m	9II06	W5381e
			SOUTH AFRICA: Cape: Near Addo	2 m	12II	W5386a,b
			SOUTH AFRICA: Cape: Hankey	1 m	12II	W5388a
			SOUTH AFRICA: Cape: Near Patensie	2 m	12II	W5389 W5390
V. menyharthii Engl. & Schinz	D	14	RHODESIA: 73 km S of Karoi	1 m	1111106	W4628a
				1 m	10II0404	W4628b
			SOUTH AFRICA: Transvaal: Wyliespoort	2 m	12II04	W&B5431d,g
				6 m	1111106	W&B5431a,b,f,i,k,r
				4 m	9II04106	W&B5431j,n-p
				2 m	7II040406	W&B5431m,q
				1 m	6II040606	W&B5431l
				1 f	14II	W&B5431h
				2 f	12II04	W&B5431c,e
V. minimum Harv.	M	14	SOUTH AFRICA: Cape: 8 km NW Grahamstown	1	14II	W5375
V. nervosum Hochst. ex A. Rich.	M	14	SOUTH AFRICA: Transvaal: 16 km W of Shina-alongubodam	1	14II	W5262
			SWAZILAND: Near Forbes Reef	1	14II	W5461
V. obovatum Harv.	M	12	SOUTH AFRICA: Natal: Mkuze Game Reserve	2	12II	W5274 W5275
			SOUTH AFRICA: Natal: 2 km N of Jozini	1	12II	W5280
			South Africa: Natal: 13-14 km SW of Jozini	2	12II	W5278 W5283
V. obscurum Thunb.	D	15	SOUTH AFRICA: Transvaal: Near Nyamundra Dam 30 km NE of Pretoriuskop	3 m	15II	W&B5424a,j,l
				1 m	13II04	W&B5424k
				1 m	10II010	W&B5424g
				1 f	9II0408	W&B5424i
			SOUTH AFRICA: Natal: Near Thousand Hills Valley	2 m	15II	W5293 W5356c
				1 m	10II010	W5356a
				1 m	9II0408	W5356b
			SOUTH AFRICA: Cape: 28 km W of King Williams Town	1 m	15II	W5372d
				3 m	13II04	W5372a,c,e
				1 m	10II0406	W5372b
			SOUTH AFRICA: Cape: 22 km W of Komga	3 m	13II04[1]	W5369a-c
			SOUTH AFRICA: Cape: 8 km NW of Grahamstown	2 m	13II04	W5384a,b
				1 m	1111040404	W5384c
				2 m	10II04106	W5384d,k
				1 m	9II0408	W5384i
				1 m	9II012	W5384g
			SOUTH AFRICA: Cape: Addo	1 m	15II	W5387b
				1 m	12II06	W5387d
				2 m	10II0406	W5387a,c
			SOUTH AFRICA: Cape: 28 km S of Avontuur	3 m	15II	W5395d,i,j
				2 m	13II04	W5395a,k
				1 m	11II0404	W5395e
			SWAZILAND: Butterworth	2 m	11II0404	W5367a,b
V. oreophilum Wiens	D	14	SOUTH AFRICA: Transvaal: Ebbe Dam	1 m	14II	W5344
			SOUTH AFRICA: Transvaal: 7 km W of Zoutpansberg summit	1 m	14II	W5311
			SWAZILAND: Near Forbes Reef	1 m	1111106	W5462
			SWAZILAND: Mbabane	1 m	14II	W5459c
				5 m	10II0404	W5458b,h-j W54591
V. pauciflorum	M	14	SOUTH AFRICA: Cape: ca 12 km NE of Worcester	1	14II	W4653
V. rotundifolium L.f.	M	14	RHODESIA, SOUTH AFRICA	3	14II	Wiens, 1975
			SOUTH AFRICA: Transvaal: Near Tshipise	1	14II	W5320
			SOUTH AFRICA: Transvaal: Pretoria	17	14II	W5436a-i, l W5455a-d,g,h,l
V. spraqueanum Burtt Davy	M	23	SOUTH AFRICA: Transvaal: 12 km S of Silberman's Post	1	23II	W5295
V. subserratum Schlecht.	D	11	SOUTH AFRICA: Transvaal: Kruger Park 2 km E of Ntokweni Range Station	6 m	9II04	W&B5427b,d,g,i-k
				2 m	7II0404	W&B5427a,h
				2 f	1111	W&B5427m,n
				1 f	9II04	W&B54271
			SOUTH AFRICA: Transvaal: 34.5 km E of Nelspruit	2 m	9II04	W5255a,c
			SOUTH AFRICA: Transvaal: 46-49 km SW of Duivelskloof	5 m	9II04	W5305c-e,h W5307
				1 m	8II06	W5305f
				2 m	7II0404	W5305a,b
			SOUTH AFRICA: Natal: Between Mkuze and Magadu	6 m	9II04	W5287e,g,h,j W5290a,b
				5 m	7II0404	W5287b-d,f,i
V. verrucosum Harv.	D	14	SOUTH AFRICA: Transvaal: 9 km E of Punda Milia	2 m	12II04[1]	W5326a,b
			SOUTH AFRICA: Transvaal: between Louis Trichardt and Messina	2 m	12II04[1]	W5326a,c
				2 m	10II0404[1]	W5326b,d
			SOUTH AFRICA: Transvaal: 50 km SW of Duivelskloof	2 m	12II04	W5304e,g
				1 m	1111106	W5304d
				4 m	10II0404	W5304b,c,f,j
				1 m	9II0406	W5304a
			SOUTH AFRICA: Transvaal: between Louis Trichardt and Pietersburg	1 m	12II04	W&B5434c
			SOUTH AFRICA: Transvaal: 5 km S of Steelpoort	1 m	1111106	W5270e
				1 m	10II0404	W5270b
			South Africa: Transvaal: near Zoutpan Experimental Farm	15 m	12II04[1]	W5298a-c W5299a,b,d W5438a-i
				1 m	10II0404	W5298d
			SOUTH AFRICA: Natal: between Mkuze and Magudu	8 m	12II04[1]	W5284a-i
				4 m	1111106[1]	W5284g,j-l
			SOUTH AFRICA: Natal: Tugela Ferry	3 m	12II04	W5351a,b,e
				2 m	10II0404	W5351c,d
			SOUTH AFRICA: Natal: 38 km N of Kranzkop	11 m	12II04	W5355a,b,d-k,m
				3 m	10II0404	W5355c,l,o
				1 m	triploid	W5355n
			SWAZILAND: 27-32 km W of Siteki	4 m	12II04[1]	W5266c W5269b,c,n
				2 m	1111106	W5269a,d
				7 m	10II0404[1]	W5266b,e-g W5269e,g,i

[1] Supernumerary chromosomes also present (see text)

[2] Monoecy is atypical (see text)

for the accumulation of adaptive gene combinations in the translocation complexes, thus giving them immediate selective value when released as floating translocations. Such complexes would have more likelihood of being conserved than raw exchanges directly exposed to selection, as would be the case in monoecious species.

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UITTREKSEL

Geslagsgekoppelde en drywende translokasiekomplekse is kenmerkend van tweehuisige (diësiese) Viscum-*spesies, maar is feitlik afwesig in die eenhuisige spesies. Die meerderheid tweehuisige spesies het vasgelegde translokasiekomplekse wat geassosieerd is met geslagsbepaling; met die manlike geslag die heterogametiese geslag. Die geslagsmultivalente is gewoonlik 'n ring van vier ⊙4 of ⊙6, maar soms ⊙8. Tweehuisige spesies sonder geslagsgekoppelde translokasies kom baie selde voor. Die meeste tweehuisige spesies is ook polimorfies vir drywende translokasie deurdat een of meer addisionele multivalente kan voorkom, wat wissel van ⊙4 tot ⊙12.*

Drywende translokasies word meer dikwels aangetref in spesies wat nie geslagsgekoppelde translokasies besit nie. Bi-chromosome is ook teenwoordig in verskeie spesies. Die verhouding manlike tot vroulike spesies is gelyk in die meeste tweehuisige spesies, maar hierdie verhouding mag verskuif; meer in die guns van die vroulikes in sommige spesies. Die hoë korrelasie tussen tweehuisigheid en translokasieheterosigositeit is 'n aanduiding dat translokasies primêr geassosieerd is met die oorsprong en vestiging van tweehuisigheid. Enige rol in die afwyking van die verwagte verhouding manlikes tot vroulikes deur "miotic drive", is heelwaarskynlik sekondêr. Geslagsgekoppelde translokasies mag tweeslagtigheid stabiliseer, deurdat dit die geslagsfaktore in noue koppeling bring. Daaropvolgende strukturele herrangskikkings binne geslagsgekoppelde translokasiechromosome mag die geslagsfaktore geneties koppel in een chromosoompaar, met die totstandkoming van drywende translokasies. Die hoë frekwensie van drywende translokasieheterosigositeit in sommige spesies dui aan dat sulke heterosigositeit ook voordelig in die plante is.

REFERENCES

AUDUS, L. J. ,1972. *Plant growth substances*. London: Leonard Hill.

BAKER, H. G., 1967. Support for Baker's Law—as a rule. *Evolution* 21: 853–856.

BARLOW, B. A. & WIENS, D., 1975. Permanent translocation heterozygosity in *Viscum hildebrandtii* Engl. and *V. engleri* Tiegh. (Viscaceae). *Chromosoma* (Berl.). 53: 265–272.

BARLOW, B. C. & WIENS, D., 1976. Translocation heterozygosity and sex ratio in *Viscum fischeri*. *Heredity* 37: 27–40.

BARLOW, B. A., WIENS. D., WIENS, C., BUSBY, W. H. & BRIGHTON, C. 1978. Permanent translocation heterozygosity in *Viscum album* and *V. cruciatum*: sex association, balanced lethals, sex ratios. *Heredity* 40: 33–38.

BAWA, K. S. & OPLER, P. A., 1975. Dioecism in tropical forest trees. *Evolution* 29: 167–179.

BAWA, K. S. & OPLER, P. A., 1977. Spatial relationships between staminate and pistillate plants of dioecious tropical forest trees. *Evolution* 31: 64–68.

BLOOM, W. L., 1977. Translocation heterozygosity, genetic heterozygosity, and inbreeding in *Clarkia speciosa*. *Evolution* 31: 256–264.

BOSE, T. K. & NITSCH, J. P., 1970. Chemical alteration of sex expression in *Luffa acutangula*. *Physiologia Pl.* 23: 1206–1211.

CHARLESWORTH, B. & CHARLESWORTH, D., 1979. A model for the evolution of dioecy and gynodioecy. *Amer. Nat.* (in press).

DANSER, B., 1941. The British-Indian species of *Viscum* revised and compared with those of South-Eastern Asia, Malaysia and Australia. *Blumea* 4: 261–319.

DARLINGTON, D. D., 1974. *Chromosome botany*. London: Allen and Unwin.

JACKSON, R. C., 1960. Supernumerary chromosomes in *Haplopappus gracilis*. *Evolution* 14: 135.

JOHN, B., 1976. *Population cytogenetics*. London: Edward Arnold.

KAPLAN, S. M., 1972. Seed production and sex ratio in anemophilous plants. *Heredity* 28: 281–285.

MULCAHY, D. L., 1967. Optimal sex ratio in *Silene alba*. *Heredity* 22: 411–423.

MULLER, J., 1925. Why polyploidy is rarer in animals than plants. *Amer. Nat.* 59: 346–353.

ROSS, M. D. & WEIR, B. S., 1976. Maintenance of males and females in hermaphrodite populations and the evolution of dioecy. *Evolution* 30: 425–441.

WEDBERG, H. L., LEWIS, H. & VENKATESH, C. S., 1968. Translocation heterozygotes and supernumerary chromosomes in wild populations of *Clarkia williamsonii*. *Evolution* 22: 93–107.

WIENS, D., 1975. Chromosome numbers in African and Madagascan Loranthaceae and Viscaceae. *Bot. J. Linn. Soc.* 71: 295–310.

WIENS, D. & BARLOW, B. A., 1973. Unusual translocation heterozygosity in an East African mistletoe (*Viscum fischeri*). *Nature New Biol.* 243: 93–94.

WIENS, D. & BARLOW, B. A., 1975. Permanent translocation heterozygosity and sex determination in East African mistletoes. *Science* 187: 1208–1209.

WIENS, D. & BARLOW, B. A., 1979. Translocation heterozygosity and the origin of dioecy in *Viscum*. *Heredity* 42 (in press).

WIENS, D. & TÖLKEN, H. R., 1979. Viscaceae. In O. A. Leistner, *Flora of Southern Africa*. 10,1: 1–59.