

POLYMORPHISM FOR OUTCROSSING FREQUENCY AT THE RAY FLORET LOCUS IN *SENECIO VULGARIS* L. III. CAUSES

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SUMMARY

Measurement of outcrossing rates of ray (female) and disc (hermaphrodite) florets of radiate plants sampled from two polymorphic populations of *Senecio vulgaris* showed that ray florets outcross at much greater frequencies. In one population from Leeds, the greater recorded outcrossing of radiate relative to non-radiate plants (Marshall and Abbott, 1984) can be entirely accounted for by the higher outcrossing of ray florets in radiate capitula while, in a second population, from Cardiff, the difference is partly explained by the same effect. It is concluded that the possession of female ray florets and the resulting functional protogyny exhibited by radiate capitula (Burt, 1977), are major causes of the reported difference in outcrossing frequency between radiate and non-radiate morphs in polymorphic populations of *S. vulgaris*. There are however, additional factors, the nature of which have not yet been determined, which may also contribute to the difference in some populations (*e.g.*, at Cardiff), if not all polymorphic populations.

1. INTRODUCTION

Measurement of outcrossing rates at the ray floret locus of *Senecio vulgaris* L. (Compositae) shows that in polymorphic populations, non-radiate plants ($T_n T_n$), which produce capitula without ray florets, generally outcross at very low frequencies (<1 per cent) whereas radiate plants ($T_r T_r$), which bear capitula with ray florets, show much greater outcrossing levels reaching 35 per cent in some instances (Marshall and Abbott, 1982; 1984). Marshall and Abbott (1982) have suggested that the greater outcrossing of the radiate morph is likely to stem from the direct effect that ray florets may have on raising outcrossing in a gynomonoeious, self-compatible composite. In contrast to the disc florets which are hermaphrodite, ray florets are female and first to open in the capitula. Thus radiate capitula are gynomonoeious and functionally protogynous (Burt, 1977) and as a consequence outcrossing should be promoted. If the possession of ray florets and the resulting functional protogyny of radiate capitula are the only factors which raise outcrossing in radiate plants, then it is to be expected that the increase would be entirely reflected in a greater outcrossing within the ray floret fraction and that there should be little or no difference in outcrossing frequency between the disc florets of radiate and non-radiate plants. Moreover, it should follow that there ought to be a ceiling value for any difference in outcrossing rates between radiate and non-radiate plants determined by the relative frequency of ray and disc florets in the capitulum.

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The aim of the study reported in the present paper was to test these hypotheses by a comparison of the outcrossing frequencies of ray and disc florets from radiate plants sampled from two wild populations of *S. vulgaris*.

2. MATERIALS AND METHODS

In practice it is difficult to differentiate exactly between seed derived from the ray and disc florets of radiate capitula since the corolla tubes are shed from the florets as the capitulum matures. However, it is straightforward to separate the seed into an "inner" and an "outer" fraction. Since the ray florets are located exclusively at the margins of the capitulum, the seed from the inner fraction will be composed entirely of seed derived from disc florets, whereas the seed in the outer fraction will contain a few seed from disc florets plus all the seed from ray florets.

Seed of radiate capitula produced by radiate plants in populations from Leeds and Cardiff sampled in June 1979 (see Marshall and Abbott, 1984) was carefully sorted into an "inner" and an "outer" fraction. This was done after a random sample of fifty mature, open pollinated plants from each population had been removed to a laboratory and stood in water. The separated seed fractions were subsequently germinated and grown to anthesis in trays of compost under glass. Since both floret fractions had been exposed to the same pollen pool in the field, the outcrossing levels at the ray floret locus associated with each fraction could be computed by dividing the frequency of heterozygotes ($T_r T_n$) found in each fraction by the frequency of the non-radiate allele (T_n) in the population of flowering plants (from Marshall and Abbott, 1984). The method employs the procedure of Allard and Workman (1963) for estimating outcrossing frequency, and assumes that the gene frequencies in the pollen pool available to radiate plants is the same as that estimated amongst the flowering populations of parent plants.

Differences between outcrossing frequencies were tested for significance using the heterogeneity χ^2 test given in Jain (1979).

3. RESULTS AND DISCUSSION

A comparison of outcrossing frequencies among the progenies of the "inner" (disc) and "outer" (mainly ray) florets of radiate capitula sampled from populations at Leeds and Cardiff, table 1, shows that ray florets outcross at much greater frequencies than disc florets. These results demonstrate that pistillate ray florets (lacking anthers) play a prominent role in promoting outcrossing in *S. vulgaris* and that their presence and the consequent functional protogyny exhibited by the radiate capitulum, are major causes of the higher outcrossing exhibited by radiate relative to non-radiate plants.

The hypothesis that the enhanced outcrossing of the radiate morph results entirely from the greater outcrossing of ray florets may be tested by a comparison of the outcrossing of disc florets of radiate plants with the outcrossing frequency exhibited by non-radiate individuals from the same population. (Estimates for the outcrossing rates of the non-radiate morph in both the Leeds and Cardiff populations are available from Marshall and Abbott, 1984). If the hypothesis is correct there should be no difference

TABLE 1

Outcrossing frequencies of (a) the "outer" and "inner" floret fractions of capitula from radiate plants, and (b) the radiate and non-radiate morphs, in populations sampled from Cardiff and Leeds, together with heterogeneity χ^2 comparisons

Population	Outcrossing frequencies	
	Cardiff	Leeds
(a) Floret fractions within radiate capitula		
Outer (ray) florets	0.225 (436)	0.063 (627)
Inner (disc) florets	0.098 (559)	0.036 (565)
$\chi^2_{(1)}$	48.16***	7.62**
(b) †Morphs in populations		
Radiate	0.137 (999)	0.060 (1316)
Non-radiate	0.007 (1046)	0.031 (1302)
$\chi^2_{(1)}$	230.6***	1.54 N.S.

N.B. Numbers in brackets represent the progeny scored.

† Morph values are from Marshall and Abbott, 1984.

between these two outcrossing rates. An examination of the data from the Leeds population, table 1, shows that the estimated outcrossing of the disc florets of radiate capitula, 3.6 per cent, is in good agreement with the estimated outcrossing frequency of non-radiate plants, 3.1 per cent ($\chi^2_{(1)} = 0.05$ N.S.). Thus the hypothesis is supported by the results from this population. In the Cardiff population, however, in which overall outcrossing level was much higher, the outcrossing rate for the disc florets of radiate plants, 9.8 per cent is considerably greater than the estimated outcrossing frequency of non-radiate plants, 0.7 per cent ($\chi^2_{(1)} = 126.0$ ***). In this population, therefore, though there is a difference in outcrossing frequency between the ray and disc florets of radiate plants this difference does not account for all of the enhanced outcrossing exhibited by radiate relative to non-radiate plants. It follows that the maximum value for a difference in outcrossing rate between radiate and non-radiate plants in the Cardiff population would be determined, only in part, by the relative frequency of ray and disc florets within the radiate capitulum.

Within the Cardiff population, other factors in addition to functional protogyny must be acting to increase the outcrossing rate of radiate plants at the ray floret locus. It is likely that within all polymorphic populations of groundsel non-radiate plants are less attractive to pollinators and are undervisited compared with the radiate morph. As a consequence the *overall* outcrossing rate of the non-radiate morph may be considerably lower than those of either the ray or disc florets of radiate plants. At first this might seem sufficient to explain the greater outcrossing of the disc florets of radiate plants compared with those of non-radiate plants at Cardiff. However, it should be noted that the additional outcrossing by the radiate morph, which stems from its greater relative attractiveness, will arise largely through outcrossing between radiate individuals alone, the non-radiate variant being seldom visited, and as such *will not be reflected in any estimate that uses the ray floret locus as a marker*. Estimates of outcrossing at the ray floret locus are based solely on crosses that occur *between* radiate and non-radiate plants. They provide neither a measure of outcrossing between radiate plants

nor one between non-radiate plants. The only circumstance under which outcrossing rate at the ray floret locus would be raised in the radiate morph as a result of its greater attractiveness is if an effect of attraction is to cause pollinators to move between morphs more frequently than expected in the non-radiate to radiate direction than in the opposite direction. An analysis of pollinator foraging patterns within polymorphic stands could test this possibility.

In a previous paper it was suggested that the greater outcrossing of the radiate morph may be due to linkage disequilibrium between alleles at the ray floret locus and alleles at other loci affecting outcrossing. It was pointed out that this may occur if specific alleles at the appropriate loci are co-adapted, in which case the disequilibrium will be maintained by selection. The possibility of the disequilibrium resulting from random local effects was ruled out by the fact that the difference between morphs in outcrossing is widespread in Britain (see Marshall and Abbott, 1984). There is, however, a third possibility, not invoked previously, which is that the disequilibrium might provide no selective advantage, but is present having become established at the time of the origin of the radiate allele in *S. vulgaris*, following introgressive hybridisation with the self-incompatible, outbreeding species *Senecio squalidus* L. (see Ingram *et al.*, 1980; Marshall and Abbott, 1980). If genes affecting outcrossing (*e.g.*, self-incompatibility alleles) were introgressed from *S. squalidus*, and were tightly linked with the radiate allele, then high outcrossing could have been closely associated with the radiate allele from the first appearance of the radiate character in groundsel. The subsequent fate of this disequilibrium would be greatly dependent on the breeding system and population structure of groundsel. The combined effects of small population size, population substructuring, founder effect and predominant self-fertilisation would all serve to minimise the rate of decay of such a disequilibrium (Hedrick, 1980). With the realisation that the ray floret polymorphism has only been widespread in Britain for a period of approximately 20–30 years (Stace, 1977) involving at most probably 60 generations (2 per annum), then, given that the additional locus or loci affecting outcrossing is tightly linked to the ray floret locus, it is not unreasonable to suppose that the disequilibrium may have decayed little over this period.

Finally, the results of the present study are worth considering in relation to the general role of ray florets in the Compositae. Ingram and Taylor (1982) have recently pointed out that in many *self-incompatible* composites in which the ray florets are positioned at the outer ring of the capitulum, the capitulum is gynomonoeious and exhibits functional protogyny though it is difficult to see how either feature might promote outcrossing. They argue that, given that self-incompatibility is undoubtedly the primitive condition in the family, there is no reason why male sterility within the ray floret fraction should have been selected as a mechanism to favour outbreeding. Instead they present evidence which suggests that male sterility is "... a developmental side of evolutionary pressure towards increased ray length..." to enhance pollinator attraction "... and may indeed be simply 'tolerated' by the taxa concerned". The present study clearly demonstrates that, though possibly of no consequence in the ancestral self-incompatible composites, the female nature of ray florets will have a profound effect on promoting outcrossing in any derived self-compatible composites.

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5. REFERENCES

- ALLARD, R. W. AND WORKMAN, P. L. 1963. Population studies in predominantly self-pollinated species. IV. Seasonal fluctuations in estimated values of genetic parameters in lima bean populations. *Evolution*, *18*, 470-480.
- BURTT, B. L. 1977. Aspects of diversification in the capitulum. In Heywood, V. H., Harbone, J. B. and Turner, B. L. *The Biology and Chemistry of the Compositae*. Vol. I, Academic Press, London, pp. 41-59.
- HEDRICK, P. W. 1980. Hitch-hiking: A comparison of linkage and partial selfing. *Genetics*, *94*, 791-808.
- INGRAM, R. AND TAYLOR, L. 1982. The genetic control of a non-radiate condition in *Senecio squalidus* L. and some observations on the role of ray florets in the Compositae. *New Phytol.*, *91*, 749-756.
- INGRAM, R., WEIR, J. AND ABBOTT, R. J. 1980. New evidence concerning the origin of inland radiate groundsel, *Senecio vulgaris* L. var *hibernicus* Syme. *New Phytol.*, *84*, 543-546.
- JAIN, S. K. 1979. Estimation of outcrossing rates: some alternative procedures. *Crop Sci.*, *19*, 23-26.
- MARSHALL, D. F. AND ABBOTT, R. J. 1980. On the frequency of introgression of the radiate (T_r) allele from *Senecio squalidus* L. into *Senecio vulgaris* L. *Heredity*, *45*, 133-135.
- MARSHALL, D. F. AND ABBOTT, R. J. 1982. Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris* L. I. Evidence. *Heredity*, *48*, 227-235.
- MARSHALL, D. F. AND ABBOTT, R. J. 1984. Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris* L. II. Confirmation. *Heredity*, *52*, 331-336.
- STACE, C. A. 1977. The origin of radiate *Senecio vulgaris* L. *Heredity*, *39*, 383-388.