Outcrossing frequencies within- and between-capitulum morphs in groundsel *Senecio vulgaris* L.

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Estimates of both within- and between-capitulum morph outcrossing in outdoor experimental plots of *Senecio vulgaris* L. show that non-radiate plants may outbreed at similar levels to radiate plants. Pure stands of both radiate and non-radiate plants were found to exhibit intra-morph outcrossing frequencies of about 17 per cent. In polymorphic plots however, pollinators appear to visit the radiate morph in preference to the non-radiate morph. This insect behaviour is shown to be related to the attractiveness of the ray florets. The implications of the results to the maintenance of the capitulum polymorphism in natural populations are discussed.

INTRODUCTION.

The capitulum polymorphism in *Senecio vulgaris* L. (common groundsel) is controlled by a single locus with codominant alleles (Trow, 1912). Radiate plants are homozygous for the *Tr* allele and non-radiates for the *Tn* allele (Hull, 1974). The *TrTn* heterozygous, short-rayed morph can be distinguished from both homozygotes. This allows the detection of outcrossing between the homozygous morphs. Several authors have investigated the level of inter-morph outcrossing in *S. vulgaris*. The first measurements, made by Trow (1912), suggested levels of outcrossing between 1 and 10 per cent. Haskell (1953) reviewed opinions concerning the breeding system in *S. vulgaris* held in the first half of this century. The consensus was that it indulged in only small amounts of outcrossing. Gibbs *et al.* (1975) also considered it to be a highly inbred species, to the point of autogamy. In experimental plots, however, levels as high as 22 per cent female outcrossing have been recorded in non-radiate lines (Campbell and Abbott, 1976). Estimates of outcrossing made within natural populations polymorphic for radiate and non-radiate capitulum types range from 1 per cent (Hull, 1974) to 20 per cent (Marshall and Abbott, 1982). Marshall and Abbott (1982, 1984) showed that in polymorphic populations the radiate capitulum morph has a greater inter-morph outcrossing frequency than the non-radiate morph.

They concluded that the capitulum polymorphism in *S. vulgaris* represents a polymorphism for outcrossing frequency at the ray floret locus. Although the methods of calculating outcrossing rates used by these authors vary, they have all utilised the ray floret locus as a marker to indicate that outcrossing had occurred. As some of them were aware, however, this procedure has the inherent weakness that the locus used to detect outcrossing is itself likely to influence the estimated level of outcrossing because insects may be more attracted to radiate plants. Furthermore, the use of the ray floret locus does not allow measurement of outcrossing rates within capitulum morphs.

The aim of the present study was to measure both within- and between-capitulum morph outcrossing rates and also to investigate features of the radiate morph such as protogyny (Burt, 1977) and rayed floret incompatibility (Warren *et al.*, 1988), which might explain the higher inter-morph outcrossing found in radiate plants by Marshall and Abbott (1982, 1984).

MATERIALS AND METHODS

Marker loci

The marker used for detecting within-capitulum morph crosses was a locus controlling bract colour. Plants homozygous for the recessive *Bg* allele produce green capitulum bracts; *BGBG* homozygotes

and *BGBg* heterozygotes have black tips to their bracts (Warren, 1987). This locus was also used as a marker for detecting between-capitulum morph crosses, in conjunction with the ray floret locus itself. These two marker loci are unlinked (Warren, 1987).

The plants

All plants used in these experiments originated from seeds collected from the wild in York. Subsequently, they were grown for three or four generations in the glasshouse in pure lines with respect to the four marker locus homozygous combinations. The experimental plants were raised under glass between May and September, 1986. On flowering, radiate plants were placed in insect-proof cages and allowed to self. This enabled plants whose rayed florets were self-compatible to be distinguished from plants whose rayed florets were self-incompatible (Warren *et al.*, 1988). Both black- and green-bract radiate plants were separated into these two groups.

Experimental plots

The basic experimental plot design throughout consisted of a central plant, in which female outcrossing was to be estimated, surrounded by a ring of eight plants which acted as potential male parents. Peripheral plants were homozygous for allele *BG* at the bract-colour locus while the central plant was homozygous for the recessive allele, *bg*. Plots were established outdoors at a variety of different sites and allowed to open-pollinate. The genotype of plants at the rayed floret locus, within plots, represented the main experimental variable. The plots were divided into two groups *with respect to the eight surrounding plants*; (1) monomorphic plots, in which potential male parents were either all radiate or all non-radiate, (2) polymorphic plots, in which the surrounding eight plants were alternately radiate and non-radiate. The central plant, from which outcrossing was estimated, was one of three possible morphs: (a) non-radiate, (b) radiate with rayed florets self-compatible or (c) radiate with rayed florets self-incompatible. All pairwise combinations of central and surrounding plants were carried out giving a total of nine plot-types. When the central plant was a self-incompatible radiate, all outer radiate plants had self-compatible rayed florets; this was to avoid the possibility of central and outer self-incompatible plants having the same pollen types. No cross made to date between self-incompatible rayed florets and

pollen from a self-compatible plant has proved cross-incompatible. Each plot design was repeated ten times at different plot locations and at various times throughout the season.

In addition, a further set of three plots was designed to investigate the insect attraction associated with rayed florets. In these plots the outer eight plants were always radiate, and the central plants were of the same three morphs as before, but their attractiveness was manipulated. When the central plant was of the non-radiate capitulum morph, artificial ray florets were attached. These were constructed from paper coloured with wax crayons and fixed to the capitulum with green p.v.c. tape so that plants appeared radiate. If the inner plants were radiate their ligules were carefully removed with scissors, leaving their rayed-floret stigmas intact, so that the plants appeared to be non-radiate.

Plants were grown in 5 cm square pots and in each plot were sunk within turf in a circle of 15 cm radius between pot centres. Thirty different plot locations were used around the Biology Department at the University of York. The number of capitula shedding pollen at any one time was restricted to five per plant. Capitula on the central plant, which had been pollinated, were tagged with green cotton. The length of time for which plots were exposed to open pollination was dependent upon collecting 100 seeds from the central plant, the number required to give a reasonably precise estimate of outcrossing frequency. This meant the collection of three capitula from non-radiate plants. Because crossing was measured separately in rayed and disc florets of radiate plants, the collection of 15 capitula was required to ensure 100 rayed floret seeds. As a result, plots with non-radiate plants in the centre were run for about one week and plots with central radiate plants for three weeks or more. After open pollination, the central plants were brought into the laboratory until their seeds ripened and could be harvested. Rayed and disc floret seeds from radiate capitula were collected separately (Warren *et al.*, 1988). Seed was stored at 16°C until December 1986 when 100 progeny per batch were grown in seed trays and their phenotypes at the two marker loci scored.

RESULTS

For each set of progeny at least 100 plants were scored and the mean percentage outcrossing was calculated over the ten replicates within plot-types. It was not always possible to obtain 100 full seed

from self-incompatible rayed florets; this was unimportant, however, as all these seeds were the result of outcrossing. In polymorphic plots using both marker loci outcrossing was further divided into that within- and between-morphs. Ninety-five per cent confidence intervals for mean outcrossing rates were calculated as follows:

For each set of ten replicate observations the variance of the replicate is

$$\text{Var}(p) = \sum (p - \bar{p})^2 / 9,$$

the variance of the observed mean of the replicates is

$$\text{Var}(\bar{p}) = \text{Var}(p) / 10,$$

and the 95 per cent confidence interval about the observed mean is

$$\bar{p} \pm t_9 \sqrt{\text{Var}(\bar{p})},$$

where p is the outcrossing frequency of each plot and $\bar{p}$ is the mean of a set of ten replicates. The sampling distribution of the replicate means was assumed to be approximately normal on account of the central limit theorem.

Mean levels of outcrossing and associated 95 per cent confidence intervals for all 12 experimental plot designs are shown in tables 1, 2 and 3.

All results for self-incompatible rayed florets confirm previous observations that these florets only set seed when crossed (Warren *et al.*, 1988). Ignoring self-incompatible rayed florets, it is clear that all monomorphic plots show a similar amount of outcrossing, about 15 per cent, with the exception of non-radiate plants surrounded by radiates.

Table 1 Outcrossing frequencies recorded from monomorphic plots

Female parents	Potential male parents	
	Non-radiate	Radiate
Non-radiate	17.8 ± 15.5	0.5 ± 0.6
Radiate (S.C.)		
Disc florets	15.2 ± 11.5	17.4 ± 10.5
Ray florets	11.2 ± 4.9	12.0 ± 7.4
Radiate (S.I.)		
Disc florets	16.9 ± 13.3	16.8 ± 8.6
Ray florets	100 (161)	100 (256)

Mean outcrossing rates and 95 per cent confidence intervals for plots in which all eight potential male parents were of a single morph. All estimates are based on 1000 progeny (100 progeny for each of ten replicate plots), except where sample sizes are shown in parentheses.

S.C. = radiate plant with self-compatible ray florets.

S.I. = radiate plant with self-incompatible ray florets.

Table 2 Outcrossing frequencies recorded from polymorphic plots

Female parents	Selfing	Within-morph outcrossing	Between-morph outcrossing
Non-radiate	79.4 ± 18.9	2.6 ± 2.8	18.0 ± 16.8
Radiate (S.C.)			
Disc florets	70.0 ± 11.8	28.9 ± 10.7	2.0 ± 1.7
Ray florets	71.4 ± 15.4	25.6 ± 14.9	3.0 ± 2.7
Radiate (S.I.)			
Disc florets	85.5 ± 8.5	11.5 ± 6.3	3.0 ± 2.9
Ray florets	0	82.4 ± 8.7 (607)	17.6 ± 7.5 (130)

Mean selfing and outcrossing rates within- and between-morphs, with 95 per cent confidence intervals, for plots in which the potential male parents consisted of four radiate and four non-radiate plants. Each treatment estimate is based on 1000 progeny (100 progeny for each of ten replicate plots), except where sample sizes are shown in parentheses.

S.C. = radiate plant with self-compatible ray florets.

S.I. = radiate plant with self-incompatible ray florets.

In this situation the outcrossing frequency falls to almost zero (table 1). The polymorphic plots all tend to show similar total female outcrossing levels of about 20–30 per cent. The radiate morph, however, appears to donate more gametes into the pollen pool than the non-radiate morph (table 2). There is also some suggestion that radiate plants in polymorphic plots have a greater female outcrossing frequency than radiates in monomorphic plots (tables 1 and 2).

Table 3 shows that most of this outcrossing can be accounted for by the insect attraction associated

Table 3 Outcrossing frequencies recorded from floral manipulation plots

Female parents	Manipulated capitula	Unmanipulated capitula
Non-radiate	7.5 ± 5.2	0.5 ± 0.6
Radiate (S.C.)		
Disc florets	1.6 ± 0.9	17.4 ± 10.5
Ray florets	2.5 ± 2.6	12.0 ± 7.4
Radiate (S.I.)		
Disc florets	3.0 ± 3.3	16.8 ± 8.6
Ray florets	100 (160)	100 (256)

Mean outcrossing rates, and 95 per cent confidence intervals, for plots in which all potential male parents were unmanipulated radiate plants. Capitulum manipulation in the female parents took the form of adding ray florets to non-radiate plants and removing ligules from radiate plants.

Unmanipulated capitulum "control" data are from table 1. All estimates are based on 1000 progeny (100 progeny for each of ten replicate plots), except where sample sizes are shown in parentheses.

S.C. = radiate plant with self-compatible ray florets.

S.I. = radiate plant with self-incompatible ray florets.

with rayed florets. Thus outcrossing levels in non-radiate plants were increased to 7.5 per cent when rays were added and were reduced to 2 to 3 per cent in radiate plants when ligules were removed from capitula.

DISCUSSION

These experiments imply that pollinating insects, when given a choice, visit radiate *S. vulgaris* in preference to non-radiate. This conclusion is manifest in several forms throughout the plot designs.

In general, the monomorphic plots (table 1) exhibit similar levels of outcrossing, which are comparable to the between-morph outcrossing frequencies of 13–20 per cent recorded by Marshall and Abbott (1982) for radiate plants. Surprisingly, monomorphic plots of non-radiate plants exhibit a within-morph outcrossing frequency of the same order as that shown by radiate plants. This observation suggests that the spread of the radiate morph of *S. vulgaris* (reviewed by Stace, 1977) has not caused an overall increase in outbreeding, but may have partitioned it in a different way. When a single non-radiate plant is surrounded by radiate plants, however, pollinating insects appear to neglect it and outcrossing falls to almost zero.

If only between-morph outcrossing in monomorphic plots is considered, the results of Marshall and Abbott (1982), that radiate plants have greater female between-morph outcrossing than non-radiate plants, are confirmed (table 1). However, the opposite result is found in polymorphic plots, where non-radiate plants show more between-morph outcrossing than radiate plants (table 2). The major differences in estimates of outcrossing rates in tables 1 and 2 can be explained by proposing two simple rules;

- (1) insects are equally attracted to all plots, irrespective of the morphs present, and
- (2) within plots, insects visit radiate plants in preference to non-radiate plants.

Rule (1) explains why, in monomorphic plots of nine non-radiate plants, outcrossing is at similar levels to that in monomorphic radiate plots (table 1). Rule (2) explains why, in polymorphic plots (table 2), radiate plants always act as male parents more frequently than non-radiate plants. Because polymorphic plots contain fewer radiate plants than monomorphic radiate plots, insect flights are concentrated within the preferred morph, and outcrossing in central radiate plants is boosted. Rule (2) also explains why when a single non-radiate

plant is surrounded by eight radiate plants it is only rarely visited by pollinators (table 1). In the reverse case where the central plant is radiate, insects are attracted from the surrounding eight non-radiate plants, and the central plant shows levels of outcrossing similar to monomorphic plots where all nine plants are radiate. These rules will not necessarily explain Marshall and Abbott's observations from natural populations because plants in the wild do not grow in plots, but have complex distributions. Furthermore, Marshall and Abbott, although restricted to measuring only between-morph outcrossing, were able to record it for all plants in the population and not just sample central plants.

Observations on polymorphic plots again show that when both within- and between-morph outcrossing are considered, the non-radiate morph is outbreeding at a similar level to the radiate morph. Earlier workers who were only able to observe between-morph crosses may, therefore, have been wrong to conclude that *S. vulgaris* is polymorphic for outcrossing frequency, with radiate plants practicing a higher level of outcrossing than non-radiate plants.

In terms of the maintenance of the capitulum polymorphism, within-morph outcrossing is irrelevant because it produces the same homozygosity at the ray floret locus as does selfing. As pointed out by Nagylaki (1974) the selective advantage of selfing (Fisher, 1941) will only occur if selfing individuals contribute to the outcrossing pollen pool. Given that total female outcrossing is equal, selection will favour the morph which contributes more to the pollen pool. The polymorphic plot results in table 2 represent just such a situation. Here both morphs exhibit similar levels of total female outcrossing, but the radiate allele is in excess in the pollen pool and is therefore favoured by gamete selection. Marshall and Abbott's (1982) results, although restricted to between-morph outcrossing, appear to show that the non-radiate morph exhibits less female outcrossing than the radiate morph. Abbott (1985) concluded that the non-radiate morph was therefore favoured by virtue of the auto-selective advantage of selfing. This conclusion is dependent on the assumption that the proportional contribution of radiate and non-radiate plants to the pollen pool is the same as the frequency of the morphs in the population. Marshall and Abbott's (1982) observations could, however, also arise as the result of the morphs showing equal levels of female outcrossing, provided the non-radiate allele was present in excess in the pollen pool.

One can only speculate about the reasons for these different conclusions, but the possibility remains that under certain conditions the two morphs will be equivalent with respect to total female outcrossing frequency and contribute equally to the pollen pool and, if all other things are equal, selection on the morphs will be relaxed. Further measurement of parameters such as relative insect attractiveness of the morphs, amount of pollen carry-over, etc and subsequent modelling might confirm this speculation.

That the inferred pollinator behaviour (rule 2) is due to the attractiveness of ray florets to insects is demonstrated by the reduction in outcrossing to the level of unmanipulated non-radiate plants when rays are removed from radiate plants (table 3). Equally, outcrossing is boosted in non-radiate plants when artificial rayed florets are added, but it fails to reach the level found for genuine radiate plants. Observations of rayed florets under ultraviolet light revealed that real rayed florets do not reflect at these wavelengths, whereas artificial rayed florets do. This suggests that the confidence trick may not be as effective to an insect's eye as to our own. Furthermore, other factors, such as scent, might also be involved. The actual pollinators on these plots are unknown, but small flies were occasionally seen to visit the plants.

Further conclusions can also be drawn from these results. (1) Self-incompatible rayed florets, only set crossed seed, but outcrossing in the radiate morph may not be significantly increased because these florets only represent about 20 per cent of the total (Richards, 1986) and they have poor seed set when cross-pollination is low. (2) Self-compatible rayed florets have similar outcrossing rates to disc florets in radiate plants, suggesting that protogyny may be having little or no effect on promoting outbreeding in *S. vulgaris*. (3) the ratio of within- to between-morph outcrossing in self-incompatible rayed florets in polymorphic plots indicates a possible slight advantage to non-radiate pollen in these florets (table 2). (4) As expected, with pollination of any form based upon only ten female parents, the 95 per cent confidence intervals are wide.

The levels of inter-morph outcrossing observed both here (tables 1 and 2) and also by Campbell and Abbott (1976) might be expected to result in a large number of short-rayed heterozygous plants in the wild. Hull (1974) and Oxford and Andrews (1977), however, have reported that such heterozygous plants are conspicuous by their rarity in natural populations. This suggests that there may be selection reducing the number of short rayed

plants or that natural levels of inter-morph outcrossing are lower than those measured in experimental plots. The latter of these two suggestions appears more reasonable. Warren (1987) found no short-rayed progeny from 2250 seed collected from natural polymorphic populations. Such low levels of inter-morph outcrossing in the wild may result from population structuring, seasonal variation or competition for pollinators. This result, however, does not imply that natural levels of intra-morph outcrossing are also low. As can be seen from table 1, inter- and intra-morph outcrossing rates may be of different orders. It is possible therefore that when levels of within-morph outcrossing are measured in the wild, they may be found to be surprisingly high.

The previously published estimates of outcrossing rates assume equal pollen production in both morphs. This was reported to be the case by Gibbs *et al.*, (1975), but Warren (1987) found frequent, and often site-specific, differences between morphs. A further assumption is that both green- and black-bract capitula are equally attractive to insects. It should also be stressed that these outcrossing measurements were made in experimental plots, which differ from natural populations in many respects, e.g., morph distributions. Other factors will also affect outcrossing frequencies in the wild. For example, the neglect of the non-radiate morph by insects when radiate plants are present may be mirrored by a neglect of *S. vulgaris* entirely when other more insect-attractive species, such as Oxford ragwort *Senecio squalidus* L., are present. This may cause the estimates of outcrossing levels reported here to be higher than those achieved in the wild.

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