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Pollen carryover and neighbourhood in *Ranunculus bulbosus*

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Abstract. The pattern of gene flow has a major influence on the spatial scale of evolutionary processes. In plant populations pollen carryover will influence the pattern of gene flow. In *Ranunculus bulbosus* L. pollen carryover was found to occur over a maximum of 5 flowers, with seed set per flower decreasing in a curvilinear fashion with increasing visit number in a sequence of visits by a bee after pollen pick-up from a donor plant. The effect of pollen carryover is to increase both neighbourhood area and neighbourhood size by two-fold.

Key words. *Ranunculus bulbosus*; gene flow; pollen carryover; neighbourhood area; neighbourhood size.

The spatial scale of evolutionary processes is determined by the pattern of gene flow, population size and the intensity of selection[1-4]. Restricted gene flow can lead to genetic substructuring within populations through random genetic drift or through selection for locally adaptive combinations of genes[5]. Consequently, knowledge of the pattern of gene flow provides information on how random and non-random forces may influence the genetic substructuring of populations. Studies of the pattern of gene flow in plants have been undertaken for more than 20 species[6-15]. But a number of these have not taken into account pollen carryover, which may significantly alter the pattern of gene flow[12,16-21]. Here we show that in the bee pollinated perennial herb *Ranunculus bulbosus* L. (bulbous buttercup) pollen carryover leads to a two-fold increase in genetic neighbourhood area and size.

Materials and methods

The effect of pollen carryover on gene flow can be assessed by calculating the area (A) and size (Ne) of genetic neighbourhoods in the presence and absence of carryover using the formulae: $A = 4\pi(\sigma_p^2/2 + \sigma_s^2)$; $Ne = Ad$; where σ_p^2 is the axial variance of pollen dispersal, σ_s^2 is the axial variance of seed dispersal and d is the density of reproductive individuals[6]. These formulae assume an outcrossing rate of 1, which is a reasonable assumption for *R. bulbosus* which is self-incompatible. The use of axial variance (which is one-half the variance of absolute dispersal distances[22]) in measures of gene flow is discussed by Kerster[23]. The development of the formulae for A and Ne is discussed by Crawford[6,22].

Pollen carryover using honey bees as pollinators was measured in a greenhouse at the Cleppa Park field station, University College, Cardiff, using pairs of self-incompatible clones. The design is depicted in figure 1. One of each pair acted as a pollen donor, the other as a recipient. A hive of bees was established in the (empty) greenhouse a few days before the clones were introduced. For each pair of clones, 30 ramets of the recipient were arranged at a distance of 5 m from the hive entrance. A spacing of 15 plants/m^2 was used. All flowers that were not newly opened were removed. One plant of the donor clone was placed between the recipient clone and the hive. The foraging sequences of bees that moved from the donor to the recipient clones were recorded, with flowers of the recipient being bagged immediately after pollination. 108 foraging sequences were recorded. Seed set was determined for each flower six weeks after pollination.

Axial variance of pollen dispersal (σ_p^2) is estimated for a natural population of *R. bulbosus* located at Rainbow Wood (grid reference ST7671), close to the University of Bath campus. The estimate is obtained from observations of 308 bee foraging runs, each observed over a maximum of 6 plants. (Personal observations have shown that the pattern of flowering in *R. bulbosus* results in a bee usually only visiting one flower per plant. The decision to follow foraging runs over a maximum of 6 flowers was based on the results of the pollen

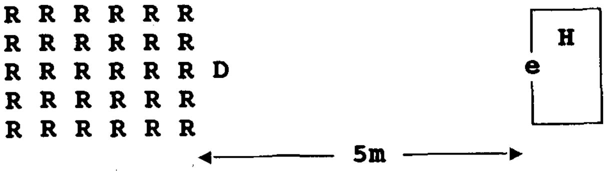

Figure 1. The design of the pollen carryover experiment. One ramet of the pollen donor clone (D) was placed between the beehive (H) and a block of 30 ramets of the pollen recipient clone (R), the front of which was 5 m from the hive entrance (e). A spacing of 15 plants/m^2 was used.

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Table. Axial variance of pollen dispersal.

Pollen recipient in foraging sequence	Proportion of seeds set in sequence (+/− s.e)	Mean flight distance (m)	Axial variance of flight distance	Weighted axial variance
1	0.5312 (0.040)	0.657	0.35	0.186
2	0.2504 (0.021)	1.244	0.76	0.190
3	0.1083 (0.009)	1.842	1.20	0.130
4	0.0595 (0.005)	2.413	1.73	0.103
5	0.0506 (0.004)	2.983	2.56	0.130
				$\sigma_p^2 = 0.739$

Axial variance of pollen dispersal (columns 4 and 5), which is estimated from observations of bee pollination events in the field (column 3) and the seed set data gathered from the greenhouse based pollen carryover experiment (column 2). Weighted estimates of axial variances for each flower are obtained as the product of columns 2 and 4.

carryover experiment described above, which showed that carryover, when estimated in terms of seed set, was effective only over the first five flowers visited in a sequence). Bees were observed to forage in approximately a straight line, allowing the distance between the pollen donor plant and recipient plants to be estimated as the sum of the distances between successive plants in a foraging sequence. σ_p^2 is estimated as the sum of the weighted axial variances for calculations assuming pollen carryover, and as the unweighted axial variance of the first recipient flower in foraging runs for calculations assuming no pollen carryover (columns 4 and 5 of the table). The weightings for these calculations are the proportions of the seeds set by each flower in a foraging sequence; these are estimated from percentage seed set data obtained from the pollen carryover experiment, and are given in column 2 of the table.

The density of reproductive individuals (d) is obtained for the study population from four randomly located 30 m^2 areas within each of which the number of reproductive individuals in 70 randomly placed 0.25 m^2 quadrats was obtained.

Axial variance of seed dispersal (σ_s^2) is estimated from the dispersal pattern of 1205 seeds from 10 plants growing in the University of Bath field research station; the seeds were trapped on sheets of muslin coated with high temperature grease that had been placed over the surface of the soil around the plants.

Results

Mean seed set per flower in recipient clones exhibited a curvilinear decrease with increasing visit number in a sequence of visits by a bee after pollen pick-up from donor clones (fig. 2 and column 2 of the table). Pollen was carried over the first five flowers in a sequence, but in no instance was carryover-when measured in terms of seed set-effective beyond the fifth flower.

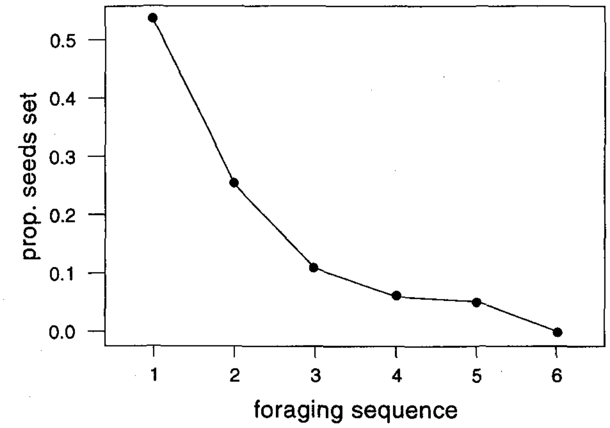

Figure 2. The proportion of seeds set per flower in recipient clones of the pollen carryover experiment (R in fig. 1) in a sequence of visits by a bee after pollen pick-up from donor clones (D in fig. 1). Foraging sequences resulted in seed set over the first five flowers in a sequence.

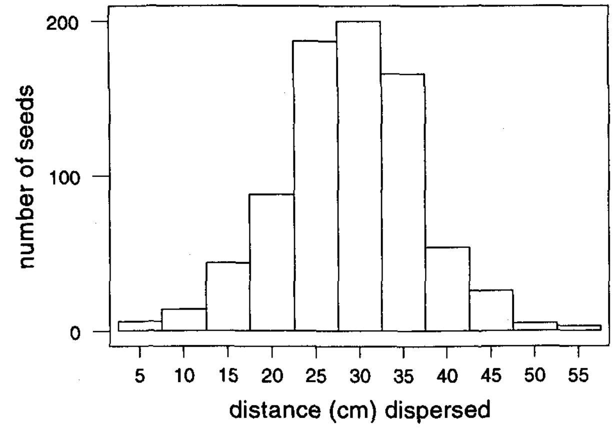

Figure 3. Seed dispersal distances for the group of seven plants described in the text.

Seed dispersal was localised, with the mean dispersal distances for the 10 plants ranging from 19.5 cm to 32.1 cm. An analysis of variance indicated that the plants can be placed into two groups with respect to the distances over which seeds were dispersed, with one group of three plants having a mean dispersal distance of 21.4 cm compared with 28.7 cm for the remaining seven plants. In both groups seed dispersal distances were approximately normally distributed; this is illustrated in figure 3 for the group of seven plants.

The axial variance of seed dispersal (σ_s^2) is estimated as 0.004. d is estimated as 17.92 plants m^2. The axial variance of pollen dispersal (σ_p^2) in the absence and presence of pollen carryover is, respectively, 0.35 and 0.739 (table). Genetic neighbourhood area in the absence and presence of pollen carryover is therefore 2.3 and 4.7, respectively; and genetic neighbourhood size in the absence and presence of pollen carryover is, respectively, 40 and 84.

Discussion

It is clear that in *R. bulbosus* pollen carryover can have a pronounced effect on the value of the estimates of neighbourhood area and size, increasing both by two-fold and thereby decreasing the importance of random genetic drift as a determinant of the genetic structure of the population. Nevertheless, even when pollen carryover is taken into account, the results indicate that the genetic structure of the study population is influenced by random genetic drift, the estimates of neighbourhood size being far below that (approximately 1000 individuals) at which virtual panmixia can be assumed[24].

The conclusions reached in this study agree with those of other studies that pollen carryover can be an important determinant of the pattern of gene flow in plant populations[12,16–20,25–27]. However, the estimates obtained of neighbourhood parameters may only approximate to those achieved in natural populations. For example, like other studies, this study has estimated potential rather than actual gene flow as it does not take into account pollen competition or the possibility that many seeds will fail to give rise to reproductively mature plants[27]. Potential and actual gene flow will be correlated, but their estimates will be very different if the probability of a successful fertilization is correlated with the distance travelled by pollen[17,28]. This situation would arise, for example, if local dispersal of pollen was less efficient than long distance dispersal due to neighbouring plants sharing incompatibility alleles or to the progeny of local matings suffering the effects of inbreeding depression and loss of heterosis[17,29–31]. Here, the measure of potential gene flow would underestimate actual gene flow. In contrast, the measure of potential gene flow would overestimate actual gene flow if pollen competition resulted in local dispersal of pollen being more efficient than long distance dispersal. It is relevant to note here that the pollen carryover experiment will have generated competition for sites on the stigma between pollen from donor and recipient plants, but as different recipient plants were members of the same self-incompatible clone (during a single pollination sequence) there would have been no competition for access to eggs between pollen from donor and recipient plants; yet competition between pollen donors for access to eggs may be common in natural populations[32]. However, for the *R. bulbosus* population studied, the axial variance of pollen dispersal would need to take a value some 9 times greater than that estimated to produce a neighbourhood size at which virtual panmixia can be assumed. There is no reason to assume that the method of estimating the variance of pollen dispersal which was employed in this study will result in a discrepancy of this magnitude.

The pollen carryover experiment demonstrated that approximately half of the seeds set following a foraging run by a bee were set on the first flower visited in the foraging sequence, and that there was an approximate 50% reduction in the number of seeds set in each subsequent flower visited (column 2 of the table). The pattern of pollen deposition on stigmas is not known for *R. bulbosus* but it may reflect the pattern of seed set, in which case this aspect of the reproductive biology of *R. bulbosus* would be similar to that found in bee pollinated *Erythronium americanum*, *Clintonia borealis* and *Diervilla lonicera*[16]. The implied deposition rate of approximately 50% of remaining pollen load being deposited on each flower in a foraging sequence may be typical of bee pollination[33].

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