How Busy Are Bees -
modelling the
pollination of clover

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Genetic markers 19
Where does the pollen effective in fertilisation come from? 20
How far do bees carry pollen? 21
Pollen carrying capacity of bees 21
Value in risk assessment 21
Bees are essential pollinators for many species of crop and wild plants that need cross-pollination for seed production (Figure 3.1). These species rely on bees to transfer pollen from the anthers of flowers of one plant to the stigmas of flowers of another. The effectiveness of bees in delivering pollen from one flower to another depends on the behaviour they adopt in foraging for nectar. In the past, the study of the consequences of their foraging behaviour was limited by the difficulty of identifying the source of the pollen that effected fertilisation and seed production, since pollen bears no obvious indication of parentage.

However, the Legume Breeding Group at IGER has now developed a range of novel genetic markers which help the investigator to define...
precisely which species the pollen comes from. In a collaborative research programme between plant breeders at IGER and pollination ecologists at the Institute of Arable Crops Research (IACR, Rothamsted), we are using these markers in a unique model system to measure pollen and gene flow mediated by bees. These new techniques allow us to examine interactions between plant and pollinator at a range of scales from single flowers to plant communities over a wide area.

**Genetic markers**

The genetic markers are all within the bee-pollinated, self-incompatible species, white clover. They include a novel red leaf marker (Figure 3.2) which is dominant over the normal green leaf type so that the progeny from crosses between these two lines have red leaves and can be visually identified. They also include five isoenzyme markers at the phosphoglucose isomerase (PGI) locus of white clover, selected within the cultivar S100 (Figure 3.3). These isoenzyme markers have the great advantage over many other markers as their selection is not associated with any other reproductive characteristics which could influence bee behaviour. The bees cannot discriminate between them. Crosses between the isoenzyme selections can be identified in the resulting seeds using a simple laboratory technique which produces a pattern similar to a supermarket barcode (Figure 3.3). In our model system, we have enclosed different species of bees on large plots of these genetically marked plants (Figure 3.4), arranged in different experimental designs, to investigate how their foraging behaviour influences seed production and paternity.

![Figure 3.3 Simplified isoenzyme banding patterns of the five white clover PGI selection lines, which are used to examine pollen transfer mediated by bees. Five selection lines of clover (green panel, labelled a - e) have a unique pattern (barcode) when leaf proteins are separated on a starch gel in an electric field and stained for activity of the enzyme PGI (phosphoglucose isomerase). When a known clover line (in this case, line a) receives pollen from any of the other (but unknown) lines, plants from the resulting seeds will display a more complex but also unique barcode pattern (examples are shown in the pink panels), which allows the unknown pollen donor to be identified. The vertical arrows indicate the direction and relative movement of the proteins in each track, with the position of the band ‘c’ shown for comparison in each case. This type of experiment allows the investigator to determine how foraging behaviour influences seed production and paternity.](image)

![Figure 3.4 Experiments were conducted in pollination cages in which plants with defined genetic markers are placed, along with hives containing the different bee species.](image)
Where does the pollen effective in fertilisation come from?

To study this, colonies of bees were enclosed with a mixed plant population having equal proportions of plants of the five isoenzyme selections. At the plant population level, the bees were thoroughly mixing the different pollens, hence they were helping to maintain genetic diversity. However, at the level of the inflorescence and the individual pod, successful fertilization was dominated by one pollen type, although usually several of the other types had also been successful (Figure 3.5). This suggests that most of the pollen effective in fertilisation comes from the last plant visited by the bee.

Are some bee species more effective pollinators than others?

Isoenzyme markers within both self-incompatible and self-fertile lines of white clover were used to demonstrate that honey bees transferred more compatible pollen to flowers than bumble bees, resulting in more seeds per pod. Honey bees visit fewer flowers on each visit to an inflorescence than bumble bees and so accumulate relatively less ineffective self-pollen onto their bodies (Figure 3.6). As they move from flower to flower within the inflorescence, honey bees are therefore more likely than bumble bees to deposit compatible cross-pollen rather than incompatible self-pollen onto the stigmas.

How far do bees carry pollen?

The distances over which bees carry pollen to other plants or other plots is an important consideration for the avoidance of
contamination of seed stocks. We assessed pollen flow between two adjacent blocks of plants, which have different isoenzyme markers, grown at different distances from each other. Pollen transfer was high over short distances (less than 1 metre) but declined to low levels when plots were more than 2 metres apart (Figure 3.7), irrespective of whether the pollen was carried by honey bees or bumble bees.

**Pollen carrying capacity of bees**

We studied carrying capacity by allowing different species of bees to visit a pollen donor, in this case with the red leaf marker, and then following the deposition of that pollen onto the stigmas of flowers of normal green leaved plants next visited by the bee. Most honey bees deposited the pollen in the next 15-20 inflorescences they visited and then only sporadically up to the 50th inflorescence. Bumble bees deposited the pollen more consistently than honey bees to the next 25 inflorescences they visited and then more sporadically up to the 90th visit. Species therefore differ in their pollen carrying capacities and their contribution to pollen dispersal.

**Value in risk assessment**

The availability of these genetic markers and our combined expertise has enabled us to develop this model system for detailed studies into how bees move pollen and genes within and between plant populations. Our studies are producing exciting new information that will help quantify pollen transfer and dispersal in insect-pollinated crop and wild plant populations. Such information is currently of particular importance in relation to risk assessment of the environmental consequences of the release of transgenic insect-pollinated crop plants.

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