An assessment of gene flow in red raspberry measured by SSRs

J. Graham

Rubus idaeus, the European red raspberry, is widely grown commercially though little is known about the interactions of commercial cultivars with wild species. Domestication has resulted in a reduction of both morphological and genetic diversity[1,2,3] with modern cultivars being genetically very similar. Future advances will require the incorporation of novel germplasm, as this lack of genetic diversity can result in vulnerability to biotic and abiotic stresses. It is important therefore to safeguard any existing diversity, and for this an understanding of what is available in the wild populations is required, and of how this is being influenced by cultivation.

Until recently, no data was available on the genetic makeup of wild species, in terms of how similar wild populations were to each other and to cultivated raspberries. Recent studies[4,5,6] now suggest that local populations are genetically distinct from cultivated raspberries and that the populations themselves have differentiated genetically and physiologically. The extent of gene flow has been inferred from genetic diversity and from morphological studies to be small, but has not been directly measured. However, this is not what would have been predicted for wild raspberry populations. As an out-breeder, with the potential for wind and insect-mediated movement of genes by pollen possibly over large distances, high levels of gene flow and little population structuring would have been expected.

Figure 1 Wild and commercial raspberry fruit.

In order to examine gene flow directly, this study set out to identify any new alleles entering one wild raspberry population via pollen flow from other wild populations or from cultivation. A system was therefore required that allowed characterisation of both alleles at each locus examined in the parental population. Once the allele status of the parental population had been determined, new alleles arising in the progeny could then be identified.

To achieve this, one wild population ('Site 12')[6] with 48 individuals was selected for study being of moderate size, readily accessible to SCRI and situated within an area of commercial raspberry cultivation. Leaf material was collected from all 48 individuals and all red fruit from the whole population was removed for seed extraction and plant germination. DNA was then extracted from each of the parental plants and from all progeny.

To identify both alleles at a particular locus, single locus polymorphic simple sequence repeat (SSR) markers were developed[7]. Repetitive DNA sequences such as short tandemly-repeated motifs account for a considerable proportion of the plant genome and

Figure 2 Glen Doll.

therefore provide an ideal marker system for this study. Changes in the number of the repeat units in the SSR, arising from mutation, results in different sized alleles at that site. These length–polymorphisms can be identified by designing primer pairs to the sequences flanking the SSR. By fluorescently labelling one of the primer pairs, PCR product identification and thus visualisation of the alleles can be achieved on an automated DNA sequencer.

From the 48 parental plants in the population, 20 produced red fruit from which seed germination occurred. A further 12 plants produced red fruit from which no seed germination occurred. The remainder either did not set fruit or the fruit did not ripen beyond the green fruit stage. Two hundred and seventy-nine plants germinated for analysis. Progeny from the same parent were analysed together.

A total of twelve loci were examined, the number of alleles identified at each of these ranged from one to five. For 19 of the 20 parental plants no new alleles were found in the progeny. For one parent, evidence of pollen flow resulting in pollination from out-with the site was detected with one new allele identified.

It is possible that other pollination events from out-with the site had occurred, but could not be identified due to all alleles being the same as those found within the site. However, given the high levels of genetic diversity found between populations in previous studies, it is possible to conclude that any underestimation of gene flow would be small.

Allele sizes were determined for the same 12 loci in the most widely grown commercial raspberry cultivars, 'Glen Moy' and 'Glen Ample'. From these, we can conclude that no pollination was detected from these raspberries and, therefore, erosion of the genetic diversity from this wild raspberry population by frequent gene flow from cultivation is unlikely.

Acknowledgements

The authors acknowledge the financial support of the Scottish Executive Environment & Rural Affairs Department.

References

[1]Haskell, G. (1960). *Watsonia* **4**, 238-255.

[2]Jennings, D.L. (1988). In: (eds.). *Raspberries & Blackberries: Their Breeding, Diseases and Growth.* Academic Press, London

[3]Graham, J.G., McNicol, R.J., Greig, K. & Van de Ven, W.T.G. (1994). *Journal of Hort. Science* **69**, 123-130.

[4]Graham, J.G., Squire, G.R., Marshall, B. & Harrison, R.E. (1997). *Molecular Ecology* **6**, 1001-1008.

[5]Graham, J.G., Marshall, B. & Squire, G. (2003) *New Phytologist* 157/3 (in press)

[6]Marshall, B., Harrison, R.E., Graham, J.G., McNicol, J.W., Wright, G. & Squire, G.R. (2001). *New Phytologist* **151**, 671-682.

[7]Graham, J.G., Smith, K., Russell, J. & Woodhead, M. (2002). *Molecular Ecology* **2**, 250-252.