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# Gene flow from cultivated to wild raspberries in Scotland: developing a basis for risk assessment for testing and deployment of transgenic cultivars

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**Abstract** In order to help establish a basis for assessing the risk associated with the testing and large-scale deployment of transgenic raspberries, wild and feral raspberry populations in Scotland were surveyed for evidence of the escape of genes introduced into raspberry cultivars by traditional breeding. The genes concerned were introduced into cultivars using traditional breeding techniques and were deployed at known times 20 to 30 years prior to the present survey. Escape of the semidominant $L_1$ gene, affecting fruit size and plant morphology, could not be detected after 30 years in test plots at the Scottish Crop Research Institute near Dundee. The recessive gene *s*, conferring spinelessness, was detected at very low frequencies (estimated at 0.004) in wild populations within the commercial production locales where cultivars carrying this gene had been introduced on a large scale beginning 21-years prior to this survey. This gene was not, however, found in any areas remote from the commercial production locales. The results of the survey indicate that escape does occur following large-scale deployment but that gene flow events are probably infrequent and spread is localized for genes having probable neutral selective value.

**Key words** Hybridization · Gene flow · *Rubus* · Spinelessness

## Introduction

The introduction of horticulturally useful traits into cultivated raspberries (*Rubus idaeus* L.) and other *Rubus* using *Agrobacterium*-mediated transformation is technically feasible (Graham et al. 1990). The deployment of an introduced gene will be considered by scientists and regulatory agencies in the light of horticultural benefits versus the environmental impact associated with its introduction into the agricultural ecosystem or its potential escape from cultivation (Hoffman 1990). The magnitude of environmental risk associated with the escape of an introduced gene from cultivation depends, in part, on the nature of the trait, the ecological consequences of the gene functioning in feral plants of the crop species or in a related wild species, the probability of escape, and the extent and rate of dispersal (Ellstrand and Hoffman 1990). These, in turn, are functions of the presence and concentrations of crop plantings and sympatric wild or weedy relatives, the reproductive systems of the crop and its relatives, and the presence of vectors for seed and pollen dispersal. Estimating risk prior to the release of a genetically engineered plant can be difficult and highly speculative without a long-term study of these parameters or an actual introduction of a new gene to obtain empirical evidence.

In several recent studies, the extent of crop-weed gene flow has been examined in various annual crops and interpreted in terms of risk management for transgenic releases (Ellstrand et al. 1989; Doebley 1990; Wilson 1990; Robert et al. 1991; Manasse 1992; Santoni and Berville 1992; Till-Bottraud et al. 1992; Kapteijns 1993; Raybould and Gray 1993; Wilson and Manhart 1993). Transgenic oilseed rape (*Brassica napus* subsp. *olifera*) lines were similar in fitness, or were less fit, when compared to conventional lines for several ecological performance parameters in field tests at multiple sites in Britain (Crawley et al. 1993). Gene flow via pollen in perennial fruit crops has been studied in order to facilitate pollination in the orchard (Jackson and Clarke 1991; Wertheim 1991), but long-term studies of gene flow between a perennial fruit crop and a sympatric wild relative are obviously difficult.

A unique combination of geography and genetic material in east central Scotland offers a convenient opportunity to assess the flow of genes in raspberry from experimental populations at a research station, and from crop

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plantations, to wild populations in the surrounding environment. Over 2000 ha of raspberries have been grown for several decades in commercial plantations in this region. The most common wild *Rubus* throughout the United Kingdom is *R. fruticosus* L., an aggregate of sterile, polyploid apomicts and fertile diploid microspecies. Hybrids between these and raspberries have not been recorded and probably do not represent an important avenue of gene escape (Raybould and Gray 1993). However, another common wild *Rubus* is the wild raspberry, *R. idaeus* (Haskell 1960; Edees and Newton 1988). Thus, the potential for gene flow from commercial plantations to the wild via seed or pollen dispersal exists at various levels throughout the country depending on the concentrations of cultivated and wild raspberries.

Raspberry breeding has been conducted at the site of the Scottish Crop Research Institute (SCRI) at Invergowrie, near Dundee, and commercial plantations and scattered stands of wild or feral raspberries are common in the countryside around the Institute. Since the breeding populations have been quite diverse, consisting, in part, of germplasm from other *Rubus* species or *Rubus idaeus* from other countries, they have contained certain genes that would not be expected in local cultivars or wild raspberries. These genes include the $L_1$ gene with a dominant phenotype that is easily recognized by elongated sepals and stipules, and extremely large fruit (Jennings 1961, 1966). $L_1$ has been incorporated in breeding populations grown in the field at SCRI since 1960.

In Scotland, it should be possible to estimate the potential for gene flow from crop plantations to the wild by determining the frequency, in wild and feral populations, of a gene that conditions spineless stems. Spinelessness is controlled by a single recessive Mendelian factor, *s* (Lewis 1939). Seedlings that are homozygous for *s* also have eglandular, rather than glandular, cotyledons. The gene *s* has been deployed since 1969 in the most popular Scottish cultivars ('Glen Clova', 'Glen Prosen', 'Glen Moy'), representing over 90% of the plantings.

Spineless plants are quite rare in native raspberry populations based on historic reports. Jennings (1964) did not detect spineless types in populations surveyed in 1957–58 from 78 sites in eastern Scotland near the major commercial production areas; nor with the exception of one plant (Haskell 1960), have they been identified in several other surveys of wild raspberries (Haskell 1960; Rousi 1965; Keep 1972; Van Adrichem 1972). Since the gene *s* was apparently absent in feral and wild raspberry populations near the eastern Scotland production areas 30 years ago (Jennings 1964), its frequency in present-day populations should give an estimate of gene flow.

The objective of the present studies was to determine the extent to which genes from the cultivated raspberry escape to feral/wild populations, either from outdoors testing at a research institute or from plantations in major production regions, in order to assist in the assessment of risk from the genetic engineering of raspberry. This was accomplished by estimating the frequency of $L_1$ and *s* in wild raspberry populations near SCRI or in other concentrations of cultivated raspberries in which these genes have been present for a known period of time.

## Materials and methods

In 1990, wild raspberry populations were surveyed for the presence of individuals with the $L_1$ phenotype or with spineless stems at 80 sites within or remote from major commercial raspberry production areas in eastern Scotland (Fig. 1 and see Table 1). All accessible sites within 2 km of SCRI were surveyed (Invergowrie vicinity). Other sites within the commercial production region ranged from 5 m to no further than 5 km from raspberry plantations. The areas surveyed 30 years ago by Jennings (1964) (Glen Esk, Glen Clova, Glen Isla, Sma' Glen, Strathbraan) were revisited and additional areas were also visited (Strathmore, the Sidlaw Hills, and three sites located north of the Grampian Mountains). The habitat for raspberries in this region is mainly restricted to roadsides along stone walls or drainage ditches where herbicides, mowing, or grazing, are not used to control the vegetation. Raspberries are also found in ungrazed fields or open woods. At each site a 500-m section of both roadsides (or a 500-m transect in woodlands) was sampled. The total number of individuals in this sampling area was estimated and spineless individuals, if present, were noted.

Since *s* can only be visualized in homozygous individuals, seedlings were raised from seeds collected at each site to determine whether there were heterozygous plants at the site which had either intercrossed or were selfed. Cotyledons of homozygous recessive (*ss*) are eglandular while *S_* seedlings have marginal glands (Jennings 1988). Five to ten fruits from each plant were collected and bulked at each site. If spineless plants were found at a site, their fruit was kept separate from that of spined plants. The seeds were extracted, scarified, cold-stratified, and germinated, following procedures described by Jennings and Tulloch (1965). The seedlings were scored for the presence of glandular hairs on the cotyledons.

## Results and discussion

Over 2800 plants at 80 sites were analyzed for their $L_1$ and *s* phenotypes. Seeds collected from these plants yielded over 21000 seedlings that were scored for glandular hairs on the cotyledons (Table 1).

There was no evidence of $L_1$ in approximately 120 plants observed within 2 km of SCRI. This gene apparently has not escaped during the 30 years of field trials, though it is possible that the somatic instability of the gene could contribute to our inability to detect it if indeed it was present in a few wild plants. Initial reports by Jennings (1966) suggested that individuals carrying $L_1$ may have associated traits, such as slight reductions in height and canes per plant, which would put them at a selective disadvantage. However, many of these disadvantageous traits have been mitigated through breeding (Jennings 1988). The numbers of plants carrying $L_1$ in trials at SCRI during any given year was probably rather small – dozens to several hundred out of thousands of plants. The low frequency of the $L_1$ allele in the experimental population certainly contributed to a small potential for escape. Furthermore, the fruits would have been harvested and removed from the field on some of the plants in yield trials, making the seeds unavailable for dispersal. Also, the importance of sanitation and cultural practices in preventing escape should not be minimized. The fencerows and unoccupied areas on and

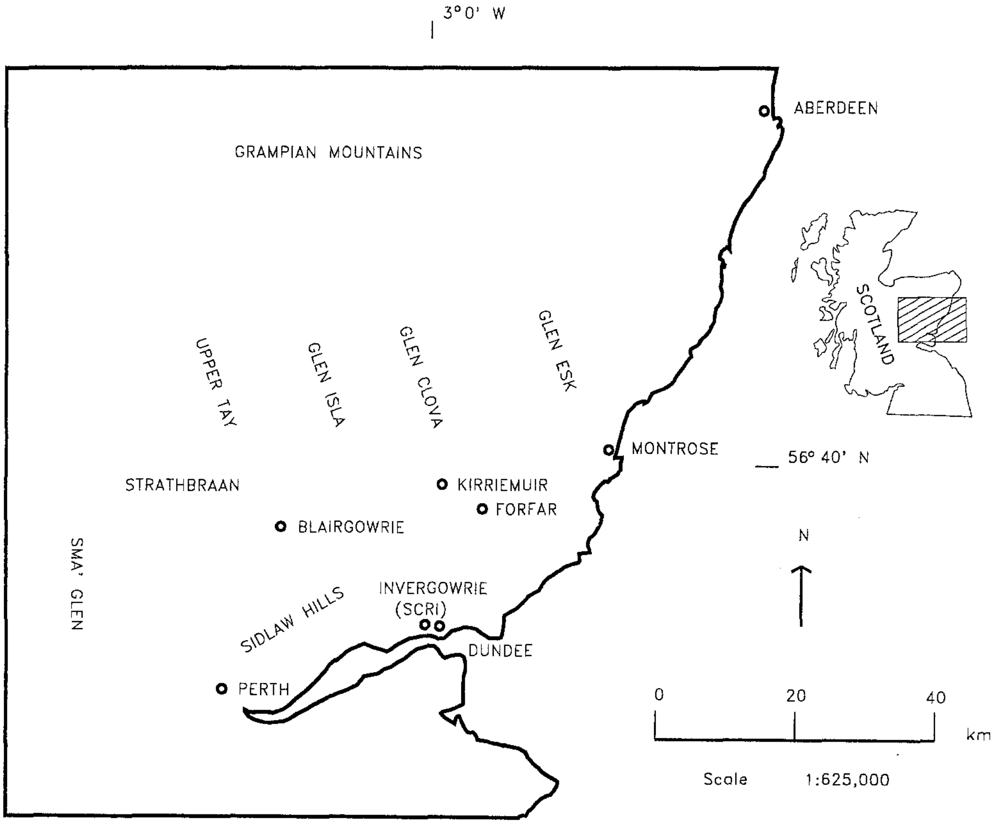

**Fig. 1** Map of east central Scotland showing the location of the Scottish Crop Research Institute, the locales of commercial raspberry plantations (Blairgowrie, Kirriemuir, Forfar, Montrose, Dundee, and Invergowrie), and areas where wild raspberries were observed and seeds were collected

**Table 1** Phenotypes of wild raspberry plants observed at sites within or remote from the commercial production region, and phenotypes of the progeny from bulked seed from spined plants

| Locale | Number of sites | Plants observed at sites | | Progeny of spined plants | |
|---|---|---|---|---|---|
| | | Spined | Spineless | Spined | Spineless |
| Sites within commercial production region | | | | | |
| Invergowrie | 13 | 251 | 4 (3 sites) | 2607 | 15 (1 site) |
| Sidlaws | 10 | 418 | 0 | 2845 | 0 |
| Kirriemuir | 7 | 480 | 2 (1 site) | 2493 | 24 (1 site) |
| Blairgowrie | 7 | 274 | 0 | 1882 | 43 (2 sites) |
| Forfar | 6 | 252 | 0 | 1363 | 0 |
| Montrose | 3 | 87 | 0 | 765 | |
| Total | 46 | 1762 | 6 | 11955 | 82 |
| Sites remote from commercial production region | | | | | |
| Glen Clova | 5 | 200 | 0 | 1134 | 0 |
| Glen Isla | 9 | 438 | 0 | 2397 | 0 |
| Glen Esk | 7 | 181 | 0 | 2015 | 0 |
| Sma' Glen | 4 | 45 | 0 | 809 | 0 |
| Strathbraan/Upper Tay | 6 | 132 | 0 | 1296 | 0 |
| Grampian | 3 | 100 | 0 | 448 | 0 |
| Total | 34 | 1096 | 0 | 8099 | 0 |

around the station are kept largely free of weedy growth through the use of mowing or herbicides. In addition, in the weedy areas around SCRI, blackberries (*R. fruticosus*) tend to more frequently occupy the disturbed and unmanaged habitats where raspberries might be expected. We observed a pattern to the distribution of *Rubus* throughout east-central Scotland in which *R. fruticosus* is the predominant species in the lower elevations and richer soils of the lowlands (from sea level to approximately 150 m) and is replaced by *R. idaeus* in the thinner soils and middle elevations (150 to 300 m) of the highland glens and mountain sides.

The spineless phenotype (putative *ss*) was observed on six plants at four sites within the commercial production region (Table 1). Apparent heterozygotes were identified at three additional sites in the production region based on the observation of eglandular (spineless) seedlings from sites where only spined plants were observed. No spineless plants or eglandular seedlings were present in sites remote from the production region. One of the putative heterozygotes was selfed and crossed with the cultivars 'Glen Clova' (*Ss*) and 'Glen Garry' (*ss*) to test for inheritance and allelism with *s* in a wild spineless plant. The selfed progeny segregated 24 spined: 11 spineless ($\chi^2$ for 3:1 is 0.77, $P<0.88$) suggesting inheritance as a single Mendelian factor. The cross with 'Glen Clova' yielded 126 spined: 31 spineless ($\chi^2$ for 3:1=2.30, $P<0.14$) and that with 'Glen Garry' yielded 25 spined: 26 spineless ($\chi^2$ for 1:1=0.02, $P<0.01$) demonstrating that this wild plant contained the same allele for spinelessness possessed by the cultivars. Assuming that the other spineless plants and putative heterozygotes also possess the same gene, then the gene for spinelessness has apparently escaped in the 20 years subsequent to its large-scale commercial deployment.

The frequency of the *s* allele remains quite low in wild raspberries in the production region; 6 of the 1768 plants observed were spineless and presumably *ss*. Progeny testing indicated the presence of at least one heterozygote at each of four sites including three sites where spineless plants were not observed (Table 1). If one heterozygote per site is assumed to have given rise to the eglandular seedlings at each of the four sites, then the allele frequency for *s* in the production region, based on six observed homozygotes and four putative heterozygotes out of 1768 plants, would be 0.0045. Because of the recessive nature of *s*, and our sampling of bulked seed lots, this may be an underestimate if the eglandular seedlings represented the offspring of more than one heterozygote per site or if offspring of heterozygotes at the other sites were not sampled. Alternatively, this estimate may overstate the actual frequency if the total number of plants at a site was underestimated because of our inability to distinguish multiple individuals that had coalesced by asexual reproduction to form a mixed stand. However, even with these inexactitudes, *s* certainly appears to be a rare allele in the wild or feral plants within the production region.

A total of 114 offspring from three spineless plants from three different sites were germinated and all but one were spineless. Since only one or two spineless plants were observed at each site, and heterozygotes apparently are also rare, the low frequency of spined offspring suggests that the feral spineless plants are mostly self pollinated or, perhaps, pollinated by nearby *ss* cultivars. Self compatibility is apparently rare in European wild raspberries based on previous surveys but is common in cultivars (Keep 1968, 1972). Cultivars are homozygous or heterozygous for a mutation ($S^{fert}$) at the locus governing incompatibility that permits pollen carrying this mutant allele to grow in styles of heterozygous ($S^+S^{fert}$) or homozygous ($S^+S^+$) self-incompatible plants (Lewis and Crowe 1958). Keep (1985) has suggested that the loci controlling self compatiblity and spinelessness are both in the the single major linkage group known in *R. idaeus*, although evidence for this is minimal and indirect (Keep 1984). The simultaneous movement of linked genes for self fertility and spinelessness might provide for a faster initial increase in the frequency of *s*. However, due to inbreeding depression, plants resulting from selfing would most likely be less fit than those resulting from cross pollination.

The gene for spinelessness apparently has not moved rapidly into wild populations since its initial deployment 20 years prior to this study. It was not detected in any sites remote from the commercial production region. Within the region, it was found at 7 of the 46 sites (15%) sampled. Six of the seven sites were within 300 m of existing or former raspberry plantations. The most distant site was approximately 2 km away from a former plantation site. Most of the sites are several kilometers from one another, so we believe they represent separate escape events.

Five of the six spineless plants observed were within 10 m of former or current raspberry fields and we judged that three of these were quite possibly asexual escapes based on their morphology and their locations in fencerows immediately adjacent to current or former fields. The exact times of these escape events during the 20 years since the deployment of *s* can not be known. However, if the average distance from the closest known sites of raspberry plantations is considered, then this gene has moved into wild populations at a rate of just a few meters per year. A gene with detrimental environmental effects escaping at this rate would pose only a limited short-term risk but, over decades, could pose a substantial risk even with such a seemingly inconsequential initial rate of escape and migration.

The extent of gene escape could also depend on either direct or indirect effects of the gene on fitness. The *s* allele in the homozygous state may confer a negative effect on fitness if the spines do indeed provide a deterrent to grazing. However, while collecting seeds and data, we observed that sheep and rabbits, the major vertebrate grazers of wild raspberries, fed readily on the first-year canes of spined plants where they were accessible. If any direct negative effect on fitness is conferred by spinelessness, it is more likely with respect to invertebrate grazers.

The presence of *s* in wild or feral plants suggests that gene flow exists between cultivated raspberries and wild populations in the commercial production region of east central Scotland. The rate of escape and apparent allele frequency of *s* have remained low over the two decades since deployment given the high frequency of *s* in the cultivated population, the abundant pollen and seed supply offered by the cultivated population, and the close proximity of the cultivated and wild populations. These results suggest that there will indeed be gene flow from transgenic raspberries deployed on a large scale in this region. However, if donor genes confer neutral or maladaptive effects on fitness in the wild setting, the extent of gene flow is likely to be quite small.

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