SHORT COMMUNICATION

Recovery of a sexual and an apomictic hybrid from crosses between the facultative apomicts *Hieracium caespitosum* and *H. praealtum*

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Abstract: Hybridisation is a rare event in facultatively apomictic species. We report the recovery of two hybrids from reciprocal crosses between the facultatively apomictic species *Hieracium praealtum* and *H. caespitosum*. Both parents were tetraploid (2n=4*x*=36). *H. caespitosum* x *H. praealtum* (CR6) was a hexaploid (2n=6*x*=54) and an apomict. The increased ploidy number is evidence of a BIII hybrid origin, having arisen from the fusion of a reduced and an unreduced gamete. In contrast, the hybrid recovered from the reciprocal cross *H. praealtum* x *H. caespitosum* (RC4) was a tetraploid and therefore probably arose as a BII hybrid from the fusion of two reduced gametes. Further evidence for this is the expression of sexuality in this plant. As apomixis in *Hieracium* is thought to be determined by a single dominant locus, a sexual plant is consistent with a model of inheritance where this represents the putative homozygous recessive phenotype. The formation of a sexual plant from the hybridisation of apomicts has potentially significant evolutionary implications. The formation of an interspecific BIII hybrid has not previously been recorded.

Keywords: apomixis; evolution; interspecific hybridisation.

Introduction

Several species of the genus *Hieracium* subgenus *Pilosella* are now recognised as major weeds of New Zealand's tussock grassland communities (Webb *et. al*., 1988). While *Hieracium praealtum* (Gochnat.) and *H. caespitosum* (Dumort) are widespread and common, *H. pilosella* (L.) is of particular concern to pastoral farmers in Central Otago and the McKenzie basin of the South Island (Duncan *et. al*, 1997; Hunter, 1991; Scott, 1985).

All three species reproduce vegetatively through stolon fragmentation, and from the formation of apomictic seed. As with most species of *Hieracium* in the subgenus *Pilosella,* seeds develop by apospory, a form of gametophytic apomixis. In the developing ovule of apomictic biotypes the products of meiosis are displaced, and typically destroyed, by one or more embryo sacs arising directly from the somatic cells of the nucellus (Asker and Jerling, 1992). As a result, at anthesis most ovules contain only unreduced or 'maternal' embryo sacs. In a small percentage of ovules functional, reduced structures remain. Reduced and maternal embryo sacs are morphologically similar, and differ principally in their chromosome complement and in the quiescence of the reduced structure after the completion of gametogenesis. Reduced embryo sacs remain intact and unaltered until either the double fertilisation of the egg and polar nuclei, or until floral senescence and the consequent degeneration of the ovule. In contrast, maternal embryo sacs do not appear to have a period of quiescence. The unreduced egg rapidly enters parthenogenic embryogenesis, proceeding in concert with the autonomous formation of an endosperm. As both reduced and maternal embryo sacs can occur among the ovules of a single plant these species are regarded as 'facultatively apomictic'.

As a result of both their relatively recent introduction to New Zealand (*H. pilosella* was first recorded late last century), and their facultatively apomictic mode of reproduction, the genetic variation within New Zealand populations of *H. pilosella*,

H. praealtum and *H. caespitosum* should theoretically be small. For example, Makepeace (1981) sampled 31 clones of *H. pilosella* from both the North and South Islands and noted that all were pentaploids (2n=5*x*=45), a cytotype common in northern Europe (Gadella, 1991) and one unlikely to be capable of forming viable gametes from meiosis. More recently however, both hexaploid and tetraploid clones of *H. pilosella* have been identified (Jenkins and Jong, 1997; Chapman and Lambie, 2000). In addition, all New Zealand populations of *H. pilosella* analysed for ISSR variation have been shown to comprise multiple genotypes (Chapman *et. al*., 2000*)*. This variation must either reflect multiple introductions, rapid evolution, or a combination of each. Mechanisms for evolution include sexual reproduction and somatic mutation. Hybridisation, a consequence of sexual reproduction, represents a potential avenue for the evolutionary divergence and radiation of these species, but to date has not been demonstrated for New Zealand *Hieracium*.

This communication describes two hybrid plants, recovered during the course of a larger research project to determine the potential for hybridisation between the common stoloniferous species of *Hieracium* in New Zealand. As hybrids between these species are difficult to distinguish morphologically, the recovery of hybrid seedlings was facilitated by using a marker gene conferring resistance to the antibiotic kanamycin, introduced into *H. caespitosum* and *H. praealtum* by *Agrobacterium*-mediated transformation (Bicknell and Borst 1994). These transgenic plants were used as pollen parents. The two hybrid plants were recovered from four capitula crosses between *H. praealtum x H. caespitosum*, and eight capitula crosses between *H. caespitosum x H. praealtum*. *H. praealtum* and *H. caespitosum* are morphologically very similar. Both form basal rosettes with few to numerous stolons and both have 0.5 - 2 cm long peduncles at flowering, bearing 5-30 yellow-petalled capitula. The primary morphological distinctions between these species are the presence of many simple hairs on the adaxial leaf surface and stellate hairs on the abaxial leaf lamina of *H. caespitosum* (Webb *et.al*., 1988).

Methods

Plant material and transformation

Plants of *H. praealtum* and *H. caespitosum* were obtained from adjacent populations in the Tekapo basin of the McKenzie country, and maintained as clones by vegetative propagation in the glasshouse. Apomixis was confirmed in both by the formation of viable seed after the emasculation of the immature capitulum (Koltunow *et. al*., 1995). Chromosome counts, using aceto-orcein staining of squashed root tip preparations revealed that both were tetraploids (2n=4*x*=36). Both formed abundant pollen of uniform size. Throughout the study plants were held in a greenhouse maintained at a day temperature of 25-30°C and a night temperature of 15-18°C. High pressure sodium vapour lamps were used to extend the natural day-length, providing a 16 hour photoperiod to promote floral induction (Yeung and Peterson, 1971). NPTII, a gene conferring resistance to the antibiotic kanamycin was introduced into *H. caespitosum* (called 'C4' because it was a tetraploid) and *H. praealtum* (called 'R4' because it was a tetraploid) by *Agrobacterium*-mediated transformation (Bicknell and Borst, 1994) using the binary vector pGA643 (An *et. al*., 1988) in strain LBA4404 (Hoekema *et al,* 1983). A transformant with a single, highly expressed copy of the introduced sequence was identified by test crossing to a recipient, self-incompatible, diploid sexual tester variety of *H. pilosella* from Europe. A clear segregation pattern of 1 resistant : 1 susceptible seedling in the progeny indicated a single functional insertion event, that the introduced sequence was heritable and penetrant and that meiosis was functional during the formation of the pollen.

Control pollination

Four capitula of *H. praealtum* were pollinated, without emasculation, at full flower opening with pollen from the transgenic plant of *H. caespitosum*. Likewise eight capitula of *H. caespitosum* were pollinated from the transgenic plant *H. praealtum*. Seed was collected, surfaced sterilised in a 1% solution of sodium hypochlorite for 50 minutes and sown onto an agar-solidified medium containing MS salts and vitamins (Murashige and Skoog, 1962), 3% sucrose and 100mg/L kanamycin sulphate.

Characterisation of the hybrids

Apomixis was tested in the hybrid seedlings by the formation of germinable seed after the emasculation of the immature capitulum. The plant was karyotyped by aceto-orcein staining of root-tip preparations, and the formation of viable pollen confirmed using Alexander's stain (Alexander, 1980). Several morphological characteristics of the parents and hybrid were recorded, including leaf shape and colour and achene dimensions. The relative abundance of simple eglandular, stellate and glandular hairs on the abaxial and adaxial sides of the leaf, peduncle, pedicels, and involucral bracts were recorded from cold frame scanning electron microscopy to confirm the intermediate character of the hybrids.

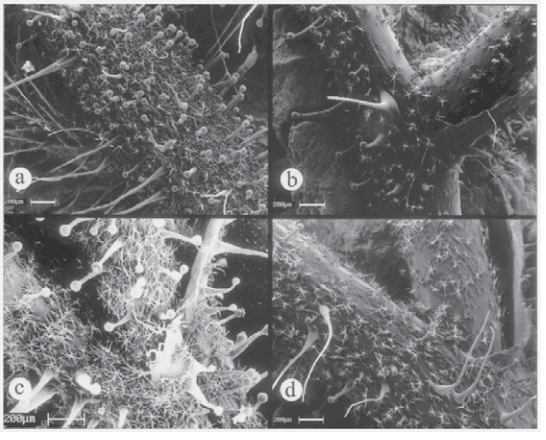

Figure 1. Electron micrographs of the stem, just below the first branch, of the two parents (*Hieracium caespitosum* and *H. praealtum*) and reciprocal hybrids, (*H. caespitosum* x *H. praealtum*; CR6) and (*H. praealtum* x *H. caespitosum*; RC4), showing variation in stellate, eglandular and glandular hair density. (a) *H. caespitosum*. Note the extremely dense stellate hairs, and scattering of both glandular and eglandular hairs. (b) *H. praealtum*. The stellate hairs are sparsely distributed across the stem. Scattered glandular hairs are visible but only one eglandular hair is recorded in the picture. (c) Hybrid CR6. Note the dense eglandular hairs similar to the maternal parent. The glandular hairs are clearly visible, a trait inherited from the maternal parent. (d) Hybrid RC4. Both eglandular and glandular hairs are relatively sparse. The presence of some long eglandular hairs is a strong paternal characteristic. Maternal and paternal traits are clearly expressed.

Results

H. caespitosum x H. praealtum (CR6)

One hybrid was recovered from a total of 180 seedlings following the pollination of four *H. caespitosum* capitula with abundant pollen of a transgenic marker plant of *H. praealtum* (R4). The plant (CR6) had 54 chromosomes, the expected complement of a hexaploid. It grew vigorously, and became taller and thicker stemmed than either parent. It formed germinable seed following emasculation, an indication that it was apomictic. The involucral bracts of the maternal parent *H. caespitosum* are covered in long dense eglandular hairs, with a scattering of glandular hairs barely visible among them. The upper peduncle of *H. caespitosum* was covered in a dense mat of stellate hairs, and many long, silver eglandular hairs; only a few scattered glandular hairs were visible (Figure 1a). In *H praealtum* (the paternal parent) the eglandular hairs were obviously confined to the central midrib of each bract, and short glandular hairs were visible scattered among them (Figure 1b). In contrast, the upper peduncle of *H. praealtum* had no eglandular hairs, but scattered glandular hairs were obvious above the dense mat of stellate hairs. CR6 bracts closely resembled the maternal parent but hairs were intermediate between both parents; long, silver eglandular and short glandular hairs were scattered above the stellate mat (Figure 1c).

H. praealtum x H. caespitosum (RC4)

A single hybrid plant was recovered from a total of eight capitula crosses which produced 360 seedlings. It was designated RC4 and had 36 chromosomes, the expected complement of a tetraploid. As with CR6 it showed hybrid vigour, being taller than either parent and with larger leaves. The bracts of the hybrid RC4 also closely resembled the maternal parent, but they were obviously more similar to *H. caespitosum* in terms of long, silver eglandular hairs. The upper peduncle of RC4 was intermediate between both parents; however the long eglandular hairs were less dense than in CR6 (Figure 1d).

In contrast to each of the parents and the hybrid CR6, the plant RC4 did not form seed following emasculation. Seeds also failed to form when the flowers were permitted to complete normal development and pollen transfer from the style to stigmatic surface was encouraged with a paint brush. To test for female sexuality, pollen was applied from a triploid aneuploid accession of *H. aurantiacum* (A3.4) from Europe. This plant was chosen as a tester because it had been previously proven to form abundant functional pollen and its genotype with respect to apomixis was known. In addition, the bright orange flowers of this species provide a valuable morphological marker for confirming paternal inheritance in *Hieracium* seedlings. Segregation in the progeny, resulting from the formation of reduced egg cells was tested by transferring 200 aseptically germinated seedlings on to a MS-based basal medium supplemented with 100 mg/L of kanamycin sulphate and scoring for the inheritance of the NPTII marker gene from RC4. One hundred and twenty-three seedlings were found to be resistant and seventy seven were susceptible to the antibiotic. A Chi squared value of 10.58 indicates that a 1:1 segregation ratio must be rejected at the 5% level. The ratio is skewed towards resistant progeny, which may have arisen through either selfing or a low level of contributing apomixis.

A separate population of seven seedlings was raised to flowering without antibiotic selection and assessed for the inheritance of paternal characteristics. All displayed paternal characteristics, including flower colours intermediate between the light yellow of RC4 and orange of A3.4. Four of the offspring were apomictic

with chromosome numbers of 2n=29, 36 and 27. Four were triploids (2n=3*x*=27), characters which are indicative of paternal inheritance and meiosis respectively.

Discussion

While our results are based on a very few crosses they demonstrate that sexual reproduction can occur between New Zealand accessions of facultatively apomictic *Hieracium* species. Furthermore, the recovery of hybrids from the reciprocal crossing of tetraploid *H. praealtum* and *H. caespitosum* demonstrated that both species can act as either a pollen or an egg parent.

The recovery of a hexaploid from the hybridisation of tetraploids indicates that this plant arose from the fusion of a reduced and an unreduced gamete (a BIII hybrid). As unreduced gametes are common in facultatively apomictic biotypes of *Hieracium,* CR6 may have arisen from the fusion of a 2n egg with a 1n sperm nucleus. Skalinska (1973) also noted the formation of BIII hybrids in progeny of the closely related species *H. aurantiacum*. In contrast, the hybrid RC4 is a tetraploid and therefore probably arose as a BII hybrid from the fusion of two reduced gametes. Gadella (1991) reported that apomixis was conferred by the inheritance of a dominant allele at a single locus in the closely related species *H. pilosella*. The formation of a sexual plant following the hybridisation of two apomicts is consistent with this model of inheritance as it represents the putative homozygous recessive phenotype. For this to happen segregation would have to have occurred in both parents, as occurs in the formation of a BII hybrid.

The potential for the formation of a sexual plant from the hybridisation of apomicts has potentially significant evolutionary implications. Although hybridisation rates in the apomictic parent accessions were relatively low, the sexual hybrid that was formed was vigorous and readily set seed following cross pollination with closely related aneuploid species. Once formed, sexual progeny plants may have the potential to act as foci for change in predominantly apomictic populations.

From this research we know that interspecific hybridisation can occur, and from field observations it appears as though this is occurring in natural populations. Plants of supposedly *H. pilosella,* but with involucral bracts intermediate between those of *H. pilosella* and *H. caespitosum* have been cultivated from field populations (H. Chapman, *unpubl. data*). Future work will aim to determine whether or not sexual plants (whether hybrids or true species) occur in the field, and then determine patterns of geographic distribution exhibited by sexuals vs apomicts. New Zealand populations of *Hieracium* provide a unique opportunity to observe patterns of microevolution within facultative apomicts. The evidence presented in this paper suggests that sexual reproduction may contribute to the unexpectedly high levels of variation found in New Zealand populations of *H. pilosella*, and explain its success as a coloniser.

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