

## RESEARCH NOTE

Interploidy interspecific hybridization in *Fuchsia*

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## Introduction

In the past, many hypotheses were put forward to explain the failure of the endosperm in interploidy crosses. In interploidy crosses, the imbalance between the genomes of different species may probably account for the breakdown of endosperm and failure of some cross combinations. The incompatibility between the genomes of parental species could be overcome by the functional  $2n$  gametes produced by meiotic nuclear restitution or by the induced chromosome doubling of one of the species. Some interspecific crosses do not meet the above criterion, especially in that genome incompatibility is not the problem in crosses between a diploid and its induced autotetraploid (Johnston *et al.* 1980). However, the correct contribution of endosperm balance number (EBN) at 2 : 1 ratio is often essential for postfertilization development of normal endosperm in plants and requires a highly specific balance of gene expression, from either the maternal or paternal genomes. Under the EBN hypothesis, the 2 : 1 proposal for many interspecific crosses, the genome of each species is assigned a specific value in the endosperm and the value may be species dependent at the same ploidy level. It is the EBN which determines the effective ploidy in the endosperm and which must be in 2 maternal : 1 paternal ratio (Johnston *et al.* 1980; Bushell *et al.* 2003). In other words, endosperm develops abnormally in interploidy–interspecific crosses when there are deviations from this 2 : 1 EBN ratio. Further, the EBN of a species may be determined by a few genes rather than the whole genome. Two species of the same ploidy but different EBN can be strongly incompatible as in  $4x$  (2EBN) *Solanum acaule* and  $4x$  (4EBN) *S. tuberosum* (Johnston *et al.* 1980). In addition, the crosses between species of different ploidy levels in the subgenus *Idaeobatus* and species in the subgenus *Eubatus* of Pacific *Rubus* (Lawrence 1986); crosses in ferns between *Dryopteris*

*intermedia* and *D. carthusiana* (Xiang *et al.* 2000) were unsuccessful.

Notwithstanding these limitations, in most crosses the excess paternal genome is associated with vigorous early growth of the endosperm with larger kernel size but shriveled seeds. The opposite is true with crosses that have maternal excess genomic endosperms. The maternal excess crosses produce plump, well formed seeds (usually smaller than the normal seeds) that give good germination. In most of the crop species, if the cross is not successful, the reciprocal crosses have resulted in viable progeny (reviewed in Haig and Westoby 1991). In this paper, an attempt was made to understand whether the use of interploidy crossings in *Fuchsia* can lead to the introgression of genetic material between species and to establish EBN arbitrary values to species based on which the success of a cross may be predicted. Taking the EBN values into account, the ploidy level of the species can be manipulated to produce the desired hybrids in *Fuchsia* breeding programme.

## Material and methods

## Crosses

In an intensive interspecific breeding programme using 15 species and three cultivars, 125 cross combinations were attempted by emasculation and hand pollination including interploidy crosses (based on the ploidy levels in parental plants). Chromosomal counts of parental species were determined using root tip squash technique (Jackson 1973).

## Plant material

The unrooted cuttings of *Fuchsia* species were obtained from the University of Auckland, Auckland; Hammett Plant Breeding Ltd., Auckland; Landcare Research, Lincoln, Christchurch (one accession of *F. procumbens*). These

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cuttings were rooted in the mist chamber in pumice, transferred to potting mixture and were grown in a glasshouse at the University of Auckland, New Zealand. The colour of sepals, petals, stamen and style was recorded using the Horticultural colour chart, issued by the British Colour Council in collaboration with the Royal Horticultural Society (Wilson 1942).

## Results and discussion

Chromosome numbers of species (table 1) indicated that amongst the species, only four were tetraploids whereas different ploidy levels were recorded in the cultivars (Talluri and Murray 2009).

In the present study, the 49 interploidy crosses made gave variable results (Talluri 2007). The plants with different chromosome numbers were capable of forming hybrids in many diploid  $\times$  tetraploid crosses such as *F. boliviana*  $\times$  *F. procumbens* (16% fruit set), *F. fulgens*  $\times$  *F. boliviana* (60% fruit set), *F. fulgens*  $\times$  *F. magellanica* (38% fruit set), *F. procumbens*  $\times$  *F. hatschbachii* (40% fruit set), *F. splendens*  $\times$  *F. magellanica* (50% fruit set), *F. triphylla*  $\times$  *F. boliviana* (43% fruit set) and *F. triphylla*  $\times$  *F. glazioviana* (8% fruit set). Taking the EBN hypothesis (Johnston *et al.* 1980) into consideration, if 2x (2EBN) the arbitrary EBN values for the diploid parental species involved in the production of above hybrids and 4x (4EBN) for polyploids were given, it confirms that at a very low-frequency crosses between species with nonmatching EBNs may also be successful. Further, in some instances interspecific hybridization between the species with same EBN can also be prevented by incompatibility between the genomes of two species (Singh *et al.* 1990). Nonetheless, the cross combination between 4x (4EBN) *F. boliviana*  $\times$  2x (2EBN)

*F. procumbens*, 2x (2EBN) *F. procumbens*  $\times$  4x (4EBN) *F. hatschbachii* and 2x (2EBN) *F. splendens*  $\times$  4x (4EBN) *F. magellanica* have shown hybrid inviability as the parental genomes failed to function in unison in these EBN mismatched hybrid progeny, thereby leading to low seed germination and high seedling mortality, a postfertilization breeding barrier. In other words, only specific combinations of the parental species produce normal viable hybrids and genetically unbalanced plants are eliminated. The cross combination of *F. splendens*  $\times$  *F. magellanica* and *F. triphylla*  $\times$  *F. glazioviana* also showed improper embryo/endosperm development with 35% and 59% of aborted seeds to the normal plump seeds in the fruit, respectively (Talluri 2007). The disharmony between parental genomes or, in some cases, improper endosperm development or endosperm failure due to imbalance in genomic imprinting between endosperm and the maternal tissue or both might lead to seed abortion (Bohs 1991; Gutierrez-Marcos *et al.* 2003).

Failure of interploidy crosses was noticed in many crosses irrespective of ploidy level of the maternal parent. Examples from this study were crosses of ‘Dr Hammett’  $\times$  *F. procumbens*, *F. glazioviana*  $\times$  *F. arborescens*, *F. glazioviana*  $\times$  *F. procumbens*, *F. glazioviana*  $\times$  *F. splendens*, *F. glazioviana*  $\times$  *F. trumpetor*, ‘Gartenmeister Bonstedt’  $\times$  *F. procumbens*, *F. splendens*  $\times$  ‘Gartenmeister Bonstedt’, *F. triphylla*  $\times$  *F. magellanica*, *F. trumpetor*  $\times$  *F. hatschbachii*, ‘Timothy Hammett’  $\times$  *F. encliandra* and ‘Timothy Hammett’  $\times$  *F. splendens*. This finding is in accord with previous work in the interspecific hybridization between pinks and both *Dianthus knappii* and carnations (Gatt *et al.* 1998). If these results are interpreted on the basis of EBN values of species, all the polyploid species mentioned in the above cross combinations possess four EBN. However, the ploidy level of cultivars varied from ‘Gartenmeister Bonstedt’ ( $2n = 33$ ), ‘Timothy Hammett’ and ‘Dr Hammett’ ( $2n = 99$ ). The imbalance between EBN of the maternal and paternal species than usually at 2 : 1 ratio for postfertilization development of normal endosperm in plants might have resulted in the failure of these crosses that are strongly incompatible. On the contrary, interspecific crosses in *Actinidia* using parents of different ploidy gave better results by choosing the genotype with lower ploidy number as the female parent (Pringle 1986).

Although the embryos in reciprocal crosses would be genetically the same, the endosperms might have different EBN ratios (Johnston *et al.* 1980). In majority of crosses: *F. arborescens*  $\times$  ‘Timothy Hammett’; *F. encliandra*  $\times$  ‘Timothy Hammett’; *F. fulgens*  $\times$  ‘Timothy Hammett’; ‘Gartenmeister Bonstedt’  $\times$  *F. procumbens*; ‘Gartenmeister Bonstedt’  $\times$  ‘Timothy Hammett’; *F. glazioviana*  $\times$  *F. procumbens*; *F. glazioviana*  $\times$  *F. splendens*; *F. magellanica*  $\times$  *F. procumbens*; *F. procumbens*  $\times$  ‘Timothy Hammett’; *F. reflexa*  $\times$  ‘Timothy Hammett’; ‘Timothy Hammett’  $\times$  *F. splendens*; *F. triphylla*  $\times$  ‘Timothy Hammett’ the reciprocal crosses were also not successful. These results confirm that the polyploids involved in the cross were having four

**Table 1.** Species and cultivars of *Fuchsia* used in this study.

Species/cultivars	Chromosome number ( $2n$ )	Section
<i>Fuchsia arborescens</i> Sims.	22	<i>Schufia</i>
<i>F. boliviana</i> Carr.	44	<i>Fuchsia</i>
<i>F. encliandra</i> Steud.	22	<i>Encliandra</i>
<i>F. excorticata</i> Linn.	22	<i>Skinnera</i>
<i>F. fulgens</i> Carr.	22	<i>Ellobium</i>
<i>F. glazioviana</i> Tamb.	44	<i>Quelusia</i>
<i>F. hatschbachii</i> Sekcja.	44	<i>Quelusia</i>
<i>F. magellanica</i> Lam.	44	<i>Quelusia</i>
<i>F. microphylla</i> Kunth.	22	<i>Encliandra</i>
<i>F. minutiflora</i> Hemsl.	22	<i>Encliandra</i>
<i>F. procumbens</i> Cunn.	22	<i>Procumbentes</i>
<i>F. reflexa</i>	22	<i>Encliandra</i>
<i>F. splendens</i> Zucc.	22	<i>Ellobium</i>
<i>F. triphylla</i> Linn.	22	<i>Fuchsia</i>
<i>F. trumpetor</i>	22	<i>Fuchsia</i>
‘Dr Hammett’	99	Cultivar
‘Gartenmeister Bonstedt’	33	Cultivar
‘Timothy Hammett’	44	Cultivar

EBN whereas the diploids had two EBN, and the crosses including the reciprocal crosses failed due to improper EBN ratios between the different species. Conversely, the reciprocal crossings between *F. triphylla* × *F. glazioviana* resulted in viable progeny despite the deviation from 2 maternal : 1 paternal EBN requirement for the proper development of endosperm. In the cross combinations such as 2x (2EBN) *F. arborescens* × 4x (4EBN) ‘Timothy Hammett’; 2x (2EBN) *F. encliandra* × ‘Timothy Hammett’; 2x (2EBN) *F. fulgens* × ‘Timothy Hammett’; 2x (2EBN) *F. procumbens* × ‘Timothy Hammett’; 2x (2EBN) *F. reflexa* × ‘Timothy Hammett’ and 2x (2EBN) *F. triphylla* × ‘Timothy Hammett’, the setback may not be the ploidy *per se* but the endosperm in these crosses is tetraploid (two genomes from female and two from male). It appears that in these crosses tetraploidy is acceptable in all tissues except the endosperm (Johnston *et al.* 1980). Thus, it is the EBN which determines the effective ploidy in the endosperm at 2 maternal : 1 paternal

ratio. The morphological description of interploidy crosses that produced plants, in some cases flowers too, is given in table 2.

One way to get around the problem of limited success in interploidy crossing may be by changing ploidy level of diploids by the application of colchicine to make them tetraploid. Induced ploidy improved fertility in crosses between *Rubus idaeus* and *R. parvifolius* and yielded better fruit set in the black and purple raspberries (Janick and Moore 1996). Future research may be directed towards induced polyploidization in the interploidy crosses in *Fuchsia*, an important pathway for gene transfer between ploidy levels. In general, even the viable hybrids showed low fertility caused by genetically unbalanced gametes resulted from random disjunction of chromosomes at anaphase I. For example, very low pollen fertility in *F. fulgens* × *F. boliviana* (9%) and *F. triphylla* × *F. boliviana* (10%) was recorded (Talluri 2011a). Further,

**Table 2.** Morphological description of interploidy hybrids in *Fuchsia*.

Cross combination	Morphological description
<i>Fuschia boliviana</i> × <i>F. procumbens</i>	This hybrid resembled <i>F. boliviana</i> in plant growth form and leaf characters. The seedlings were less vigorous and short lived (died within a month after germination showing tip-burning symptoms).
<i>F. fulgens</i> × <i>F. boliviana</i>	This interesting hybrid had intermediate plant growth form between the two parental species. The plants were vigorous and the flowers were entirely different from both parental species with varied (number and size) stamens in the segregated population. The sepals were rose madder to rose bengal in colour; the petals had rose madder to carmine colours and stamens were positioned at the same level as the style.
<i>F. fulgens</i> × <i>F. magellanica</i>	This hybrid resembled <i>F. magellanica</i> in plant growth. The plants were vigorous and had entirely different flowers of both the parental species. The flowers were medium in size with rose madder 23 coloured sepals, rose bengal 25/1 petals and eight stamens located at three different levels. The filament was rose bengal 25/3 in colour with whitish anthers and exerted stamens. The style and stigma were rose madder 23/2 in colour and this hybrid had profuse long flowering period.
<i>F. procumbens</i> × <i>F. hatschbachii</i>	The seedlings resembled <i>F. hatschbachii</i> in plant growth form and leaf shape. These seedlings had very stunted growth, tip burning, die back symptoms and died within a month after germination.
<i>F. splendens</i> × <i>F. magellanica</i>	This hybrid also showed a stunted plant growth habit and was short lived (died within two months of transplantation).
<i>F. triphylla</i> × <i>F. boliviana</i>	This hybrid showed entirely different characters and did not resemble either parental species. The plants were shrubs and vigorous, and had great variation in its flower characters. The pendulous flowers varied in sepals colour in the segregating population from geranium lake 20/1, carmine 2/1 faded towards tips to rose madder 23/1. The petals varied in colour from magenta 27/1 at the top, carmine 21/1 at the bottom; crimson 22/1 with top cyclamen purple 30 to rose madder 23/1 with rhodamine purple 29/1 at the top. The flowers had six stamens and the colour of the filament varied from crimson 22/2 to geranium lake 20/3. The anthers were sulphur yellow 1/3 and in one of the segregating population, anthers and stamens were modified as coloured petals. The style was crimson 22/2 to whitish and the stigma colour varied from whitish, magenta 27/1 to geranium lake 20/3.
<i>F. triphylla</i> × <i>F. glazioviana</i>	This hybrid showed the combination of both the parental species in its leaves and plant growth form. The hybrid had pigmentation in the leaves like in the other hybrids that involves <i>F. glazioviana</i> as one of the parents.

pollen sterility was observed in the cross combinations *F. fulgens* × *F. magellanica* and segregating population of *F. triphylla* × *F. boliviana*. Studies on meiosis together with the presence of different shapes and sizes of pollen grains in *Fuchsia* proved indirectly that unreduced gametes are the products of first division meiotic nuclear restitution. In these interploidy hybrids, during meiosis, in addition to univalents and bivalent; trivalents and quadrivalents were also recorded (Talluri 2011a,b).

In conclusion, interploidy crosses are associated with genetically unstable progeny. The failure of interploidy crosses is not directly responsible for prefertilization barriers but postfertilization reproductive isolation of species involved in the breeding programme. Interestingly, in *Fuchsia*, crosses between species with different ploidy levels were successful in some of the cross combinations and failure of interploidy crosses was seen in many crosses irrespective of the ploidy level of the maternal parent. The imbalance between EBN of the maternal and paternal species than usually at 2 : 1 ratio for postfertilization development of normal endosperm in these hybrid plants might have resulted in failure of these crosses and are strongly incompatible in addition to disharmony between the genomes of both species.

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