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## Chromosome numbers in the genus *Libertia* (Iridaceae)

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**Abstract** Chromosome numbers have been determined for seven species of *Libertia* from New Zealand, Australia, and South America. An extensive polyploid series, ranging from diploid with  $2n = 2x = 38$  through  $2n = 12x = 228$ , has been found.

**Keywords** *Libertia*; Iridaceae; chromosome numbers; polyploidy

### INTRODUCTION

*Libertia* Sprengel is the only genus of the Iridaceae native to New Zealand. A small number of species are also found in Australia and New Guinea (2), and South America (c. 3). In the most recent revision of the genus for New Zealand, Moore (1967) recognised four species: *L. pulchella*, *L. grandiflora*, *L. peregrinans*, and *L. ixioides*. Chromosome numbers were determined from meiotic metaphase I preparations for all of these species and a complex polyploid series was recorded (Hair et al. 1967; Moore 1967). The lowest number ( $2n = 38$ ) was found in *L. pulchella*, *L. grandiflora* and *L. peregrinans* had  $2n = 114$ , and *L. ixioides* had  $2n = 228$ . For the non-native species, Sanders et al. (1983) obtained counts

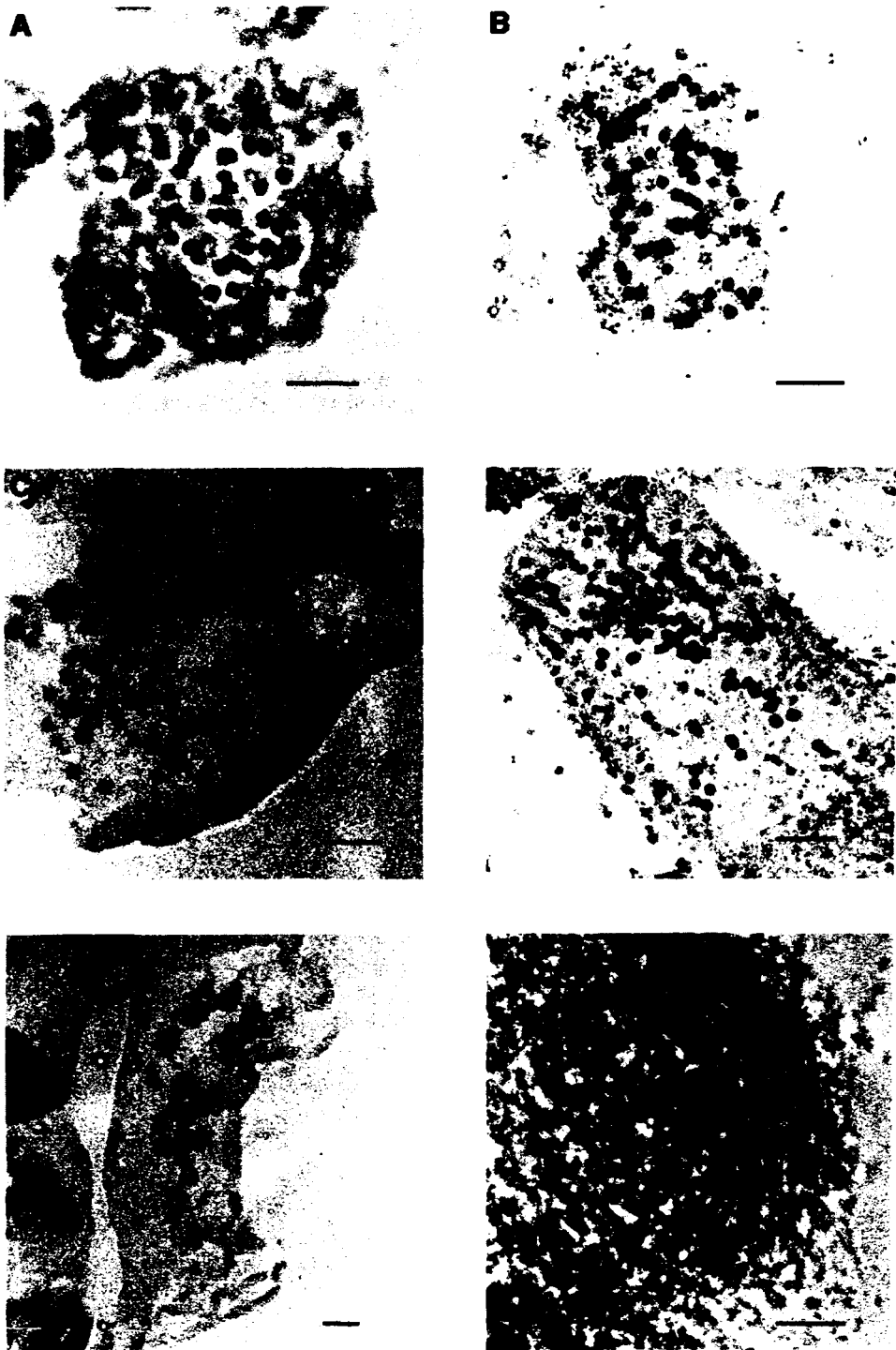
of plants described as *L. chilensis* (syn. *L. formosa*), from the Juan Fernandez Islands off the west coast of Chile, and found a number of  $n = 57$  ( $2n = 6x = 114$ ). This differs from the record of M. I. Dawson (unpubl. data) on material from mainland Chile which had  $2n = 4x = 76$ . Kenton & Heywood (1984) studied two accessions of the genus, but had difficulty getting accurate counts, noting that both the extremely small size and the presence of large satellites on some chromosomes made this difficult. However, they reported two counts, one of  $2n = c. 72$  for *Libertia ixioides* from Chile (presumably *L. formosa*, as *L. ixioides* is a New Zealand endemic) and the other of  $2n = c. 230$  for *L. grandiflora* from New Zealand. All the previously published chromosome numbers of the genus *Libertia* are listed in Table 1.

As part of a comprehensive revision of the genus in New Zealand, which will be published separately, we have re-examined the chromosomes of all the New Zealand species, using material from their complete geographic range. Several rhizomatous or dwarf taxa with uncertain affinities were included. Four other species from Australia or South America were also examined.

### MATERIALS AND METHODS

Plant material was obtained from a variety of sources and is listed in Table 1. Voucher specimens have been deposited in the herbarium of the Auckland Institute and Museum (AK).

Root tips were collected between 10.00 a.m. and 12.00 noon and placed immediately in a saturated solution of paradichlorobenzene (PDB) overnight at 4°C. The root tips were then fixed in ethanol:glacial acetic acid (3:1) overnight at 4°C and then transferred to 70% ethanol for storage at –20°C until required. Roots were hydrolysed in 1 M HCl at 60°C for 10 min, and transferred to Feulgen stain for at least 1–2 h, in the dark. The stained meristematic tip was then excised on a slide and macerated in a small drop of FLP orcein (Jackson 1973), heated, and



**Fig. 1** Mitotic chromosomes of **A**, *L. caerulescens* ( $2n = 2x = 38$ ); **B**, *L. formosa* ( $2n = 4x = 76$ ); **C**, *L. grandiflora* ( $2n = 6x = 114$ ); **D**, *L. ixioides*  $\times$  *L. grandiflora* (artificial hybrid) ( $2n = 9x = 171$ ); **E**, *L. ixioides* (rhizomatous) ( $2n = 12x = 228$ ); **F**, meiotic chromosomes of *L. formosa* ( $2n = 4x = 76$ ). Bars = 10  $\mu$ m.

squashed. Several cells (preferably more than 5) from more than one root tip were counted to ensure that a consistent count was achieved.

For meiotic analyses, immature flower buds were collected from inflorescences before the two long bracts had opened and were fixed in absolute ethanol:chloroform:glacial acetic acid (6:3:1) at 4°C for 24 h. The buds were then transferred to 70% ethanol for short-term storage at 4°C or long-term storage at -20°C. Anthers were dissected out and gently squashed in a drop of FLP orcein on a slide, gently heated, and squashed. Chromosomes were photographed with Pan F film, the images were digitised with a Nikon LS1000 film scanner, and whole images were manipulated with Adobe Photoshop 3.0.5.

## RESULTS

### New Zealand taxa

*Libertia* chromosomes are small (1.0–2.0 µm) and too numerous for meaningful karyotype analysis (Fig. 1, Table 1). However, in most species the counts obtained were clear and unambiguous. *L. pulchella* was consistently diploid ( $2n = 2x = 38$ ) throughout New Zealand, including larger plants from Northland. All material of *L. grandiflora* (including dwarf forms from Nelson and Marlborough and rhizomatous plants from Wellington) and *L. peregrinans* (excluding inland forms from Nelson) was hexaploid ( $2n = 6x = 114$ ). Material of *L. ixioides* from both the North and South Islands was found to have 228 chromosomes, although definitive counts were difficult. Occasionally counts were obtained of between 220 and 230 chromosomes. Populations of *L. ixioides* from East Cape, which are rhizomatous, and others with large capsules were all found to have 228 chromosomes.

Interesting numbers were obtained for some *L. peregrinans* populations from inland north-west Nelson. These were nonaploid and had  $2n = 9x = 171$ . Our unpublished molecular data suggest that they are interspecific hybrids between *L. peregrinans* and *L. ixioides* (Blanchon 1999).

Artificial hybrids between *L. ixioides* and *L. grandiflora* were found to be nonaploid ( $2n = 9x = 171$ ) (Fig. 1D).

### Other *Libertia* species

Chromosome counts of Australian and South American species are given in Table 1. Tasmanian specimens of *L. pulchella* were found to be diploid

( $2n = 2x = 38$ ), as were accessions of the South American species *L. caerulescens*. The Australian *L. paniculata* and Chilean *L. formosa* were both found to be tetraploids ( $2n = 4x = 76$ ) (Fig. 1).

### Meiosis

Meiosis was observed in *L. caerulescens* and *L. formosa*, and in both cases only bivalent formation was seen (Fig. 1F). Despite numerous attempts, it was not possible to observe meiosis in other species.

## DISCUSSION

The results reported here confirm the counts of Moore (1967) and Hair et al. (1967) and extend our knowledge of the chromosome status of a significant number of additional *Libertia* populations in New Zealand. We have identified putative interspecific hybrids from the north-west Nelson region with a nonaploid chromosome number and suggest that they are F1 hybrids between *L. peregrinans* and *L. ixioides* (Blanchon 1999). We also report the first counts for Australian material of *L. pulchella* and for *L. paniculata*, as well as for the South American species *L. caerulescens*. Our counts of  $2n = 76$  for *L. formosa* agree with that of M. I. Dawson (unpubl. data) but differ from those of Sanders et al. (1983). The latter reported *L. formosa* from the Juan Fernandez Islands as a hexaploid, which suggests that the Juan Fernandez plants are either a different species or a hexaploid cytotype of the mainland *L. formosa*. Kenton & Heywood (1984) counted a plant of *L. ixioides* (Forst.f.) Sprengel from Chile as being tetraploid ( $2n = c. 72$ ). This plant is probably referable to *L. formosa*, because *L. ixioides* is a New Zealand endemic, and plants previously placed in *L. ixioides* (Forst.f.) Sprengel or *L. ixioides* Klatt from Chile have been placed in *L. formosa* Graham. Thus, we have a complex of species with a wide range of chromosome numbers based on  $x = 19$ . Goldblatt & Takei (1997) considered that the basic chromosome number for *Libertia* was  $x = 19$ , based on the work of Hair et al. (1967), but noted that the base number for taxa in South America needed to be verified. It now appears clear that there is but a single basic number in the genus.

The distribution of species with different chromosome numbers is interesting with diploids being found in New Zealand, Australia, and South America, tetraploids only in Australia and South America, and the hexaploids and dodecaploid being confined to New Zealand. This pattern of distribu-

**Table 1** Chromosome numbers of *Libertia* taxa studied and previously published counts

Species NZ Taxa	2n	Source/origin/reference	Voucher
<i>L. pulchella</i>	2n = 2x = 38	Omahuta Forest, Northland	
		Matauraua Plateau, Northland	AK 240209
<i>L. grandiflora</i>	2n = 6x = 114	Waitakere Ranges, Auckland	
		Mt Ruapehu, Central North Is	AK 240210
		Mt Burnett, NW Nelson	
		Mt Brown, Westland	
		Unknown, Talisman Nurseries	
		Hair et al. (1967)	
		Moore (1967)	
		Mt Manaia, Northland	AK 240211
		Karekare, Auckland	
		Hicks Bay, East Cape	AK 240212
Dwarf form	2n = 12x = 228	Rimutaka Summit, Wellington	
		Unknown, Auckland University	AK 240213
		Pelorus Bridge, Marlborough	AK 240214
		Takaka Hill, Nelson	
		Mt Burnett, Nelson	AK 240215
		Marlborough (Platt Nurseries)	AK 240216
		Marlborough (Talisman Nurseries)	
		Butterfly Creek, Wellington	AK 240217
		Rhizomatous	
		Rhizomatous	
<i>L. peregrinans</i>	2n = 12x = 228 2n = 12x = c. 230 2n = 6x = 114	Petone, Wellington	
		Hair et al. (1967)	
		Moore (1967)	
		Moore (1967)	
		Kenton & Heywood (1984)	
		Foxton Beach, Manawatu	AK 240218
		Charleston, Buller	AK 240219
		Hokitika, Westland	AK 240220
		Chatham Island	
		Mt Albert Research Centre	
Inland form	2n = 9x = 171	Hair et al. (1967)	
		Moore (1967)	
		Spey River, Nelson	
		Cobb Valley, Nelson	AK 240221
<i>L. ixiooides</i> x <i>L. grandiflora</i>	2n = 9x = 171	Artificial hybrid	AK 240222
<i>L. ixiooides</i>	2n = 12x = 228		
		Hihi Manganui, Northland	
		Kawaka Ridge, Waikato	AK 240223
		Taranaki (ex Platt Nurseries)	AK 240224
		Rai Valley, Marlborough	
		Pohara, NW Nelson	
		Tummil River, Nelson	
		Dunedin Botanical Gardens	
		Hair et al. (1967)	
		Moore (1967)	
Rhizomatous		East Cape (ex Platt Nurseries)	AK 240225
Rhizomatous		Unknown, Albert Park, Auckland	AK 240226
<b>Foreign Taxa</b>			
<i>L. caeruleascens</i> (South America)	2n = 2x = 38	Bay Bloom Nurseries, Tauranga	
		Auckland University Grounds	AK 240227
<i>L. formosa</i> (South America)	2n = 4x = 76	Dunedin Botanical Gardens	AK 240228
		Auckland University Grounds	AK 240229
		Landcare, Lincoln	AK 240230
<i>L. ixiooides</i> (Chile)	2n = 6x = 114 2n = 4x = c. 72	Sanders et al. (1983)	
		Kenton & Heywood (1984)	
<i>L. pulchella</i> (Australia/ New Guinea)	2n = 2x = 38	Tasmania	AK 240231
<i>L. paniculata</i> (Australia)	2n = 4x = 76	Hollard Gardens, Taranaki	AK 240232

tion of chromosome races, where there is also a preponderance of species at the higher ploidy levels, is found in several other genera in New Zealand such as *Ranunculus* (Rendle & Murray 1989) and *Pratia* (Murray et al. 1992). The origin of the hexaploid *Libertia* species in New Zealand is somewhat problematical. Hexaploids cannot be produced by somatic doubling of diploids and there are no tetraploids to produce the required unreduced gametes which could combine with reduced gametes to produce hexaploids. It is possible that previously established tetraploids have become extinct in New Zealand or, alternatively, that there were hexaploids in Australia which are now extinct but could have been the source of the New Zealand hexaploids. One or more of the New Zealand hexaploids or their ancestors presumably gave rise to the two dodecaploids, via the fusion of unreduced gametes, hybridisation of hexaploids followed by chromosome doubling, or autopolyploidy of one hexaploid species.

This high level of polyploidy seen in *Libertia* is not uncommon in the Iridaceae. Many genera, including *Iris*, *Moraea*, *Crocus*, and *Gladiolus*, have extensive polyploid series (Goldblatt & Takei 1997). Within the tribe Sisyrinchieae, *Sisyrinchium* has extensive polyploidy, ranging from diploid to dodecaploid, although base numbers for the genus are not certain. *Olsynium* and *Orthrosanthus* also have polyploid species.

The base number of  $x = 19$  for *Libertia* is high. Most other genera in the Sisyrinchieae have base numbers of  $x = 8-12$ , although *Diplarrhena* has  $x = 16$  and *Sisyrinchium* can have  $x = 17$  (Goldblatt & Takei 1997). There are three possible explanations for this high basic number. The first is that the whole genus is polyploid; species presently considered to be diploid may be, in fact, tetraploid. Goldblatt & Takei (1997) suggested that in the early evolution of the Iridaceae, there was a burst of polyploidy followed by descending dysploidy in many genera. They considered ascending dysploidy to be uncommon in plant evolution, a view shared by Stace & James (1996) in their paper on the Lobelioideae. Supporting this idea is the view that *Libertia* is considered to be morphologically the most primitive genus in the Sisyrinchieae and has been used as an outgroup for phylogenetic analyses of the tribe (Goldblatt et al. 1990). A second possible explanation is that *Libertia* is of hybrid origin, a cross between a  $x = 9$  taxon and a  $x = 10$  taxon, followed by chromosome doubling. This would mean that the Sisyrinchieae all had low base chromosome num-

bers, and that the high number for *Libertia* is derived. A third possibility is that ancestral *Libertia* species with  $x = 9$  or 10 crossed to form allotetraploids, or one doubled to form an autotetraploid (gaining or losing a chromosome pair), and then became extinct, leaving only the tetraploid descendants. Testing of these hypotheses could be difficult, although a study of the NOR chromosomes of *Libertia* and its relatives (as done by Gatt et al. (1998) for *Dahlia*) could indicate whether the genus is basally diploid or tetraploid.

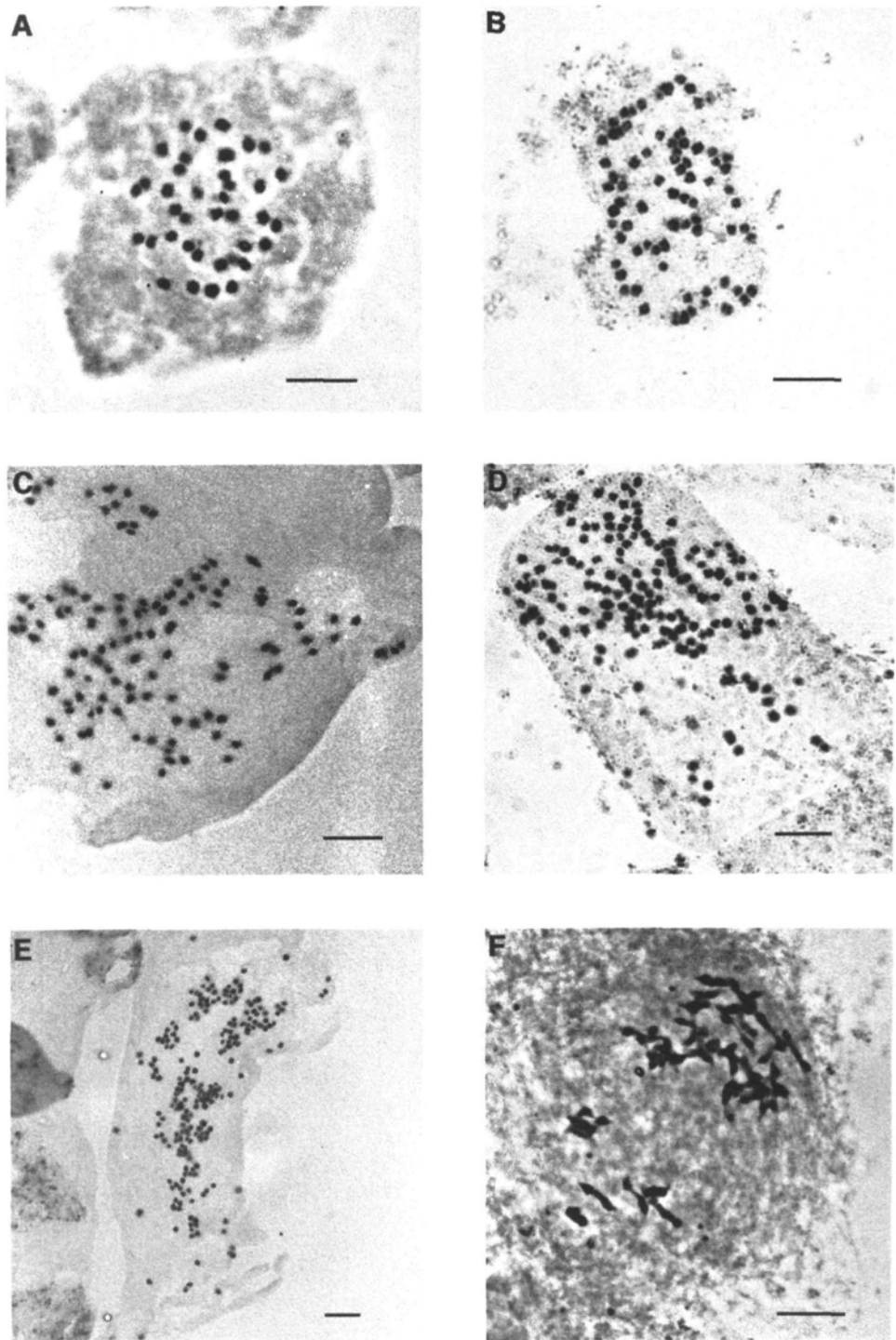
## ACKNOWLEDGMENTS

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**Fig. 1** Mitotic chromosomes of **A**, *L. caerulescens* ( $2n = 2x = 38$ ); **B**, *L. formosa* ( $2n = 4x = 76$ ); **C**, *L. grandiflora* ( $2n = 6x = 114$ ); **D**, *L. ixiooides* × *L. grandiflora* (artificial hybrid) ( $2n = 9x = 171$ ); **E**, *L. ixiooides* (rhizomatous) ( $2n = 12x = 228$ ); **F**, meiotic chromosomes of *L. formosa* ( $2n = 4x = 76$ ). Bars = 10  $\mu$ m.