NEW CHROMOSOME COUNTS IN *NARCISSUS* CULTIVARS

PETER BRANDHAM

The evolution of flowering plants is a subject that has fascinated scientists for many years. In the wild, new forms appear and old ones die out by a process of variation and natural selection that is well-documented but agonisingly slow in relation to our own lifetimes. The process of development and change in cultivation is much more rapid, because of the greatly enhanced levels of selection. Of course, this is not real evolution, but in some circumstances it can reflect the slower process that occurs in the wild, and is consequently a fruitful field of study. Cultivars of *Narcissus* are ideal subjects in which to examine the development of a decorative plant under the influence of mankind, because the genus has exploded in cultivation over the past 150 years from a few species and a small number of derived cultivars to the slightly larger number of species and the more than 22,000 cultivars that have been named from them. Many cultivars are now extinct, but the rest, which must all be propagated vegetatively as clones to maintain their identity, are of all ages and are ideal material for evolutionary studies, since the older and middle-aged ones comprise the intermediate steps that we can still follow today.

My own interest is in the chromosomes of these plants, and I have shown that the major pattern of chromosomal change in cultivation has been in the numbers of chromosome sets in them, i.e. their level of polyploidy. Most species and the early cultivars are diploid with two sets of seven (2n = 14), but later cultivars have more sets, with larger and more vigorous triploids (three sets; 2n = 21) arising from diploids probably in the 1840s, and even larger tetraploids (four sets; 2n = 28) arising from triploids in the 1890s.

Generally, diploid hybrids are reasonably fertile, except where the parents of the hybrid are very different in their chromosome structure, in which case meiosis in the hybrid fails and causes sterility (meiosis is the process of chromosomal pairing and separation that precedes the formation of pollen and egg cells, which normally have half the chromosome number of the plant producing them).

Triploids are highly sterile, because of their irregular meiosis, but they are nevertheless vital steps in the production of tetraploids. The occasional sex cell produced by a triploid avoids meiosis, it has the same number of chromosomes (three sets) as the plant that gave rise to it and is viable, unlike the remainder of the sex cells which contain an unsuccessful attempt to reduce the three sets by half and are not viable. In an appropriate cross, these unreduced triploid gametes will fuse with the single set of chromosomes in the sex cell of a diploid plant to produce a tetraploid seed with four sets. This will grow into a fertile plant that might be the starting point for further breeding. Plants with more than four sets are known, but are uncommon and I have suggested elsewhere that they are above the optimum level of polyploidy as far as size and vigour are concerned and are consequently not selected by breeders as being worth growing.

This article presents the results of my recent investigations on the chromosome numbers of *Narcissus* cultivars, most of which were not known previously, and discusses their significance. It is known from my earlier researches that most new *Narcissus* cultivars in Divisions 1-3 are tetraploid with 2n = 28 chromosomes or thereabouts (since meiosis in these plants can produce viable sex cells with a range of chromosome numbers from 13 to 15, giving a potential viable range from 26 to 30 in the progeny). They are mostly fertile enough to be used in breeding programmes with a good chance of success, so it was not considered profitable to examine the chromosomes of many more of them, since little more significant information would be generated. Members of some other *Narcissus* divisions are at an earlier stage of chromosome evolution, because they comprise largely diploids and the triploids derived from them, with fertile tetraploids remaining uncommon or, in the case of Division 5, unknown. It is of great importance to the daffodil breeders who are interested in these divisions to know the chromosomal constitution of their plants, because of the high frequency and low fertility of triploids and the need to adopt different techniques when using them in breeding programmes. The plants studied were therefore drawn largely from these other divisions, which have been found to be of greater interest as far as their chromosomes are concerned, or they were examined at the request of *Narcissus* breeders who had found that some varieties were unexpectedly sterile or unexpectedly fertile, or believed to be so. In these cases it was hoped that chromosome studies could shed some light on the degree of fertility or sterility of the plants.

Results

Chromosome numbers of 78 varieties were obtained from Feulgen-stained root-tip squashes and are listed in Table 1. A few of them (e.g. 'Lilac Charm', 'Roberta Watrous', 'Silver Bells') are confirmations of earlier results, but the rest are new. They will be discussed below under several headings, starting with chromosome number in ascending order.

2n = 14

These diploid hybrids would be expected to be reasonably fertile, with the exception of 'Elka', in which differences in the structure of the two sets of chromosomes indicate that the plant is a wide hybrid, i.e. the parents are genetically very dissimilar. This plant is unlikely to be fertile.

The seventeen Division 3 and 9 plants (poets and similar short-cup varieties) that were examined arrived with a claim that they had diploid seed parents. If this were so, they would be expected to be diploids or triploids, depending on the level of polyploidy of the pollen parent. Of these plants, seven were diploid as expected, but ten were tetraploid. Both parents of the latter would certainly be tetraploid rather than diploid, since tetraploid progeny from diploid / triploid crosses are very rare and difficult to obtain, so the information on the parentage of these plants is largely in error.

2n = 21

These triploid plants can originate in either of two ways. One is from solely diploid stock, with non - reduction during meiosis in one parent providing fourteen chromosomes and normal meiosis in the other providing seven. Triploids arising in this way are larger and more vigorous than their diploid siblings and are therefore often preferentially selected. In Divisions 1-3 the first step towards polyploidy occurred via triploid formation over 150 years ago, but in some of the other divisions it is happening now (see below).

The other way in which triploids can arise is from a diploid / tetraploid cross. This will often happen when a cultivar (usually tetraploid) is crossed with a species (usually diploid).

Triploids are the cause of some confusion as far as their fertility and breeding potential are concerned. In the database of cultivars and their origins issued on CD-ROM by the American Daffodil Society, many cultivars known to be triploid are recorded as fertile, on the basis that there is at least one known record of their being a successful parent. This is misleading, since all triploids are in fact highly sterile. Meiosis in them is usually chaotic, with poor chromosome pairing followed by an unsuccessful attempt to divide an odd number of chromosome sets by two, resulting in widespread abortion of pollen and ovules. The only viable gametes that triploids can produce are those which are non-reduced and have the same chromosome number as the plants that gave rise to them. These non-reduced gametes give some chance of breeding success, particularly if the triploid is used as the male parent in a cross.

Although highly sterile, triploids are thus not totally sterile, although it is clearly incorrect to call them fertile. In *Narcissus*, absolute sterility can occur only in those Division 4 varieties in which the double flowers have no functional anthers or stigmas.

2n = 22

These plants are triploids with an extra chromosome and come from tetraploid / diploid crosses with a chromosomal irregularity in the tetraploid parent. This uncommon chromosome number confirms that 'Suzy Dee' and 'Suzy's Sister' (both 2n = 22) are probably clonal, as suspected by their breeder, Brian Duncan, with one being a vegetative mutation or sport of the other.

'Silver Bells' was also found to have 2n = 22, agreeing with earlier records. This number fits the pedigree of at least one of the progeny of 'Silver Bells'. A very rare non-reduced gamete (22) from 'Silver Bells', fusing with a haploid gamete (7) from a normal diploid, has produced 'Lapwing' (tetraploid plus one = 29) and possibly others.

2n = 24

In most species and cultivars of *Narcissus* the basic chromosome number is x = 7, giving diploids with 2n = 14, triploids with 2n = ± 21 and abundant tetraploids (among the cultivars) with 2n = ± 28. In *N. tazetta* and its allies the basic number is x = 10 or 11, giving diploids with 2n = 20 or 22 and triploids with 2n = 30 or 33. Tetraploids with 2n = 40 or 44 are unknown. Members of the *N. tazetta* alliance can hybridise with the rest of the genus, but because of irregular pairing during meiosis the occurrence of 10 and 7 together in the hybrids makes them sterile, e.g. in varieties with 2n = 10 + 7 = 17, 2n = 10 + 7 + 7 = 24, or 2n = 10 + 10 + 7 = 27. The only exceptions are a few allotetraploids such as 'Matador', with 2n = 10 + 10 + 7 + 7 = 34. These have normal meiosis, since the chromosomes in one set of ten pair with those of the other set of ten and the two sets of seven do likewise. They are highly fertile and are proving to be excellent parents, e.g. of 'Falconet' and 'Hoopoe', which are both hybrids of 'Matador' and *N. jonquilla*.

'Explosion', 'Falconet' and 'Hoopoe' are sterile triploids with 2n = 24, and have *N. tazetta* chromosomes in them (2n = <u>10</u> + 7 + 7), in common with the well-known Division 12 varieties ‘Jumblie’, ‘Quince’ and ‘Tête-à-Tête’. Although sterile, they could be equally successful commercially, with their combination of vigour and multiple-flowered habit.

2n = 27, 28, 29

These plants are tetraploids with some variation in chromosome number (aneuploidy) resulting from meiotic irregularity in one or both parents, which does not always divide the 28 chromosomes into two equal groups of 14. Aneuploid sex cells with 13 or 15 chromosomes are sufficiently viable to survive into the progeny. Euploid and most aneuploid tetraploids are sufficiently fertile to be used in breeding programmes. An exception is in Division 4, in which the double flowers often produce hardly any pollen because the stamens are converted largely or completely into petaloid structures. They frequently have no functional styles or stigmas either, and are thus completely sterile.

‘Castanets’ is an interesting unique plant. Its count of 2n = 27 is not (4 x 7) - 1 as in many other cases, but 10 + 10 + 7, which is the first record of this chromosome combination. Its probable chromosomal origin is 10 from one parent and 10 + 7 from the other. I would guess its ancestry as *N. tazetta* (2n = 20) crossed with a *tazetta* hybrid, either a very rare fertile one with 2n = 10 + 10 + 7 + 7 = 34 or a sterile diploid hybrid with 2n = 10 + 7 = 17 with meiotic non - reduction. Being an unbalanced allotriploid, ‘Castanets’ would be highly sterile, but in common with many other triploids it could see horticultural success through vegetative propagation. As it has 2 doses of *N. tazetta* chromosomes (2n = <u>10</u> + <u>10</u> + 7 = 27), rather than the single dose in ‘Explosion’, ‘Falconet’, ‘Hoopoe’, ‘Jumblie’, ‘Quince’ and ‘Tête-à-Tête’(which have 2n = <u>10</u> + 7 + 7 = 24), it should resemble the *N. tazetta* alliance more closely than they do.

2n = 30

Two plants were found with this chromosome number. ‘Interloper’ is an aneuploid tetraploid with 2n = (4×7) + 2, formed as above, as a result of meiotic irregularity in its parent(s), and it is presumably adequately fertile for breeding purposes. ‘Soleil d’Or’ (2n = 3 × 10) is yet another example of a triploid. I have been working on the possibility that the names ‘Soleil d’Or’ and ‘Grand Soleil d’Or’ are not synonyms, but that two forms of this well-known cultivar might exist, a diploid with 2n = 2 × 10 = 20 and a larger and more vigorous triploid with 2n = 3 × 10 = 30, but so far no diploid has appeared. It still seems that the two names are used indiscriminately for the same plant, but the search for the diploid continues. If any reader can spare a small bulb of this variety, especially if it is long - established, I would be pleased to receive it in the continuing search for the elusive diploid.

B chromosomes

These are in several of the plants surveyed. They carry few genes, if any, and are transmitted erratically to the progeny. As far as breeding is concerned, they are neutral in their activity, having little or no effect on fertility, but conferring no advantages either. The highest ever record of their numbers in *Narcissus* was found here in triploid 'Martinette', which has three. The variety would be good material for teaching the occurrence and genetics of these largely parasitic chromosomes and its ancestors would also be worth investigating to determine their inheritance pattern.

Variable counts

Two chromosome chimeras were found:- The first of these, 'Dimple', has 14 chromosomes in most of the roots and is thus diploid, but one root had 28 chromosomes throughout and indicates the presence of a tetraploid sector in the plant. The variety has been reported to have breeding difficulties, which could result from the juxtaposition of diploid and tetraploid cells in the flower. Somatic doubling of chromosome number arising in this way is a regular but rarely-reported phenomenon, and can lead to the production of a sport if an entire offset is affected.

The other variety with chromosome number irregularities was 'Inca'. This is an aneuploid tetraploid with 27 chromosomes, but some cells with less than half of this number were seen. These were initially dismissed as broken cells which had lost some chromosomes during the preparation procedure, as happens frequently, but it was then noticed that they all had 13 chromosomes, and not a range of numbers as would be expected. A cell in this plant had unusually lost 14 of its chromosomes but kept growing and dividing to comprise a significant part of the cell complement. This is a very unusual occurrence, since chromosome loss usually results in early abortion of the affected cells. Again the juxtaposition of cells with 13 and 28 chromosomes could explain the reported breeding difficulties encountered in 'Inca'.

Divisions 5-7

These divisions, which mainly comprise miniatures, are attracting increasing interest among breeders. The chromosome numbers of a good sample of them are known and are shown in Table 2. I have often pointed out that the key to producing large numbers of vigorous, successful cultivars is to exploit polyploidy, as was done in Divisions 1-3 (albeit unconsciously) when triploids appeared in the 1840s, with tetraploids arising from them in the 1890s. Triploids are sterile (see above), but are vital steps in tetraploid production. Table 2 shows that Divisions 6 and 7 contain ample numbers of tetraploids, from which new cultivars can be raised with relative ease, but Division 5 is another matter. Prior to the present work, Division 5 was known to comprise only diploids and sterile triploids derived from them, i.e. it was at the stage of development occupied by Divisions 1-3 during the second half of the nineteenth century. Now there is a major breakthrough. 'Lapwing' and 'Mission Bells' are tetraploid and are likely to be fertile. If they are crossed together, I am confident that they will release a host of vigorous fertile cultivars in this division for the first time.

Acknowledgements

I am greatly obliged to the following people for providing the material for this study:- J. Blanchard, M. Bradbury, B. Duncan, D. Du Plessis, Sir F. Harrison, S. Kington and R. Scamp

Table 1. Chromosome numbers of 78 *Narcissus* cultivars

Cultivar	Division	Chromosome No. (2n)
‘Akepa’	5W-P	21
‘Androcles’	4W-W	28
‘Atholl Palace’	4W-Y	27
‘Bagatelle’	1Y-Y	14
‘Ballykinler’	3W-GYR	27
‘California Rose’	4W-P	27
‘Castanets’	8Y-O	27
‘Clare’	7Y-Y	14
‘Cotinga’	6W-P	27
‘Delta Flight’	6W-W	28
‘Diatone’	4W-P	28
‘Dimple’	9W-O	14/28
‘Dorchester’	4W-P	28
‘Dunkery’	4Y-O	28
‘Elizabeth Ann’	6W-GWP	27
‘Elka’	1W-W	14
‘Explosion’	8Y-O	24+1B
‘Eye Level’	9W-YYO	14
‘Fairy Footsteps’	3W-GGW	14
‘Fairy Glen’	3W-GWW	14
‘Falconet’	8Y-R	24
‘Fanad Head’	9W-GGR	14
‘Favor Royal’	3W-GYR	28
‘Georgie Girl’	6W-GYP	29
‘Gresham’	4W-P	28
‘Hill Head’	9W-GGR	14
‘Hollypark’	3W-GYR	28
‘Honey Bells’	5Y-Y	21
‘Hoopoe’	8Y-O	24+0-1B
‘Huon Pride’	4W-W	28
‘Ice Wings’	5W-W	22
‘Inca’	6YW-WWY	27/13
‘Innovator’	4O-R	28
‘Interloper’	6W-O	30
‘Intrigue’	7Y-W	21
‘Jingle Bells’	5W-Y	21
‘Joybell’	6W-Y	29
‘Kiltonga’	2W-YYR	28

‘Jamestown’	3W-GYY	28
‘Kokopelli’	7Y-Y/Y	21
‘Ladies Choice’	7W-W	28
‘Lapwing’	5W-Y	29
‘Lilac Charm’	6W-GPP	27
‘Lincolnshire Double White’	4W-YYR	14
‘Martinette’	7Y-O	21+3B
‘Mary Lou’	6W-W	28
‘Marzo‘	7Y-Y	21
‘Melbury’	2W-P	28
‘Midget’	1Y-Y	14+1B
‘Mission Bells’	5W-W	28+1B
‘Mizzen Head’	9W-GYY	14
‘Moon Jade’	3W-GWY	28
‘Moon Tide’	3W-YOO	29
‘Nether Barr’	2W-GRR	28
‘Oryx’	7Y-W	21
‘Parterre’	2W-Y	28
‘Patois’	9W-GYR	14
‘Port Patrick’	3W-GOR	29
‘Prototype’	6Y-YPP	28
‘Reggae’	6W-P	27
‘Ringhaddy’	3W-GYO	28
‘Roberta Watrous’	7Y-GYP	21
‘Ruby Rose’	4W-R	27
‘Satin Blanc’	7W-W	28
‘Sheer Joy’	6W-W	28
‘Silver Bells’	5W-W	22
‘Soleil d’Or’	8Y-O	30
‘Spalding Double White’	4W-YYR	14
‘Sunday Chimes’	5W-W	21
‘Sun Disc’	7Y-Y	14
‘Suzie Dee’	6Y-Y	22
‘Suzie’s Sister’	6Y-Y	22
‘Tamar Valley Double White’	4W-Y	14
‘Top of the Hill’	3W-GYY	28
‘Top Notch’ × *N. jonquilla*	7Y-Y	21
‘Torr Head’	9W-GYR	14
‘Tracey’	6W-W	21
‘Whipcord’	7Y-O	21

Table 2. Records of chromosome numbers in *Narcissus* cultivar Divisions 5, 6 and 7. New or confirmed records are in italics.

Division 5	2n	Division 6	2n	Division 7	2n
‘Akepa’	*21*	‘Bartley’	21	‘Aurelia’	21
‘April Tears’	14	‘Beryl’	21	‘Baby Moon’	14
‘Arish Mell’	21	‘Bushtit’	21	‘Baby Star’	14
‘Auburn’	21	‘Charity May’	21	‘Bell Song’	21
‘Celestial’	21	‘Cornet’	21	‘Bobbysoxer’	22
concolor (triandrus)	14	*‘Cotinga’*	*27*	‘Bolton’	21
‘Happy Easter’	21	‘Cyclone’	28	‘Bunting’	22
‘Hawera’	14	*‘Delta Flight’*	*28*	‘Buttercup’	21
‘Honey Bells’	*21*	‘Delta Wings’	28	‘Cherie’	21
‘Horn of Plenty’	21	‘Dove Wings’	21	*‘Clare’*	*14*
‘Ice Chimes’	22	*‘Elizabeth Ann’*	*27*	‘Cora Ann’	21
‘Ice Wings’	*22*	‘Elrond’	28	‘Dickcissel’	21
‘Jingle Bells’	*21*	‘Fairy Wings’	14	‘Divertimento’	21
‘Johanna’	21	‘February Gold’	21	‘Eland’	21
‘Lapwing’	*29*	‘February Silver’	35	‘Golden Incense’	21
‘Lemon Heart’	21	‘Foundling’	27	‘Golden Perfection’	31
‘Liberty Bells’	21	‘Garden Princess’	28	‘Golden Sceptre’	21
‘Mission Bells’	*28+1B*	*‘Georgie Girl’*	*29*	‘Gripshover’	14
‘Moonshine’	21	‘Gimli’	27	‘Hesla’	21
‘Niveth’	21	‘Golden Cycle’	21	‘Hillstar’	28
‘Rippling Waters’	21	‘Golden Lacquer’	28	*‘Intrigue’*	*21*
‘Samba’	21	*‘Inca’*	*27/13*	*‘Kokopelli’*	*21*
‘Shot Silk’	21	*‘Interloper’*	*30*	*‘Ladies Choice’*	*28*
‘Silver Bells’	*22*	‘Itzim’	21	‘Lanarth’	21
‘Sunday Chimes’	*21*	‘Jack Snipe’	21	‘Limequilla’	28
‘Sydling’	21	‘Jana’	21	‘Lintie’	21
‘Thalia’	21	‘Jenny’	21	*‘Martinette’*	*21+3B*
‘Thoughtful’	21	‘Jetfire’	21	*‘Marzo’*	*21*
‘Tresamble’	21	*‘Joybell’*	*29*	‘Mockingbird’	21
‘Tuesday’s Child’	21	‘Larkelly’	36	‘Ocean Spray’	21
‘Ucluluet Gem’	21	‘Larkwhistle’	21	‘Orange Queen’	14
		‘Lilac Charm’	*27*	*‘Oryx’*	*21*
		‘Little Witch’	21	‘Parcpat’	21
		‘March Sunshine’	21	‘Penpol’	21
		‘Mary Kate’	27	‘Pink Step’	28
		‘Mary Lou’	*28*	‘Pipers Barn’	20
		‘Mini Cycla’	14	‘Pipit’	21
		‘Nymphette’	27	‘Polnesk’	21

	‘Orange Glory’	28	‘Porthchapel’	21
	‘Peeping Tom’	21	‘Quick Step’	28
	‘Perfect Spring’	21	*‘Roberta Watrous’*	*21*
	‘Prototype’	*28*	*‘Satin Blanc’*	*28*
	‘Reggae’	*27*	‘Shah’	28
	‘Roger’	37	‘Skylon’	22
	‘Sextant’	28	‘Snow Bunting’	21
	‘Sheer Joy’	*28*	‘Sugarbush’	21
	‘Snoopie’	28	‘Sundial’	14
	‘Suzie Dee’	*22*	*‘Sun Disc’*	*14*
	‘Suzie’s Sister’	*22*	‘Suzy’	21
	‘Tracey’	*21*	‘Sweetness’	21
	‘Trena’	21	‘Sweet Pepper’	21
	‘Urchin’	28	‘Tittle Tattle’	21
	‘Woodcock’	29	*‘Top Notch’ x N. jonquilla*	*21*
			‘Trevithian’	21
			‘Verdin’	21
			‘Waterperry’	21
			‘Whipcord’	*21*
			‘Yellow Prize’	28

3 diploids (9.7%)	2 diploids (3.8%)	7 diploids (12.1%)
26 triploids (83.9%)	22 triploids (41.5%)	42 triploids (72.4%)
2 tetraploids (6.4%)	26 tetraploids (49.0%)	9 tetraploids (15.5%)
	3 pentaploids (5.7%)	
Total 31	Total 53	Total 58