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The involvement of *Narcissus hispanicus* Gouan in the origin of *Narcissus bujei* and of cultivated trumpet daffodils (Amaryllidaceae)

by

B.J.M. Zonneveld

NCB Naturalis, section National Herbarium of The Netherlands, Leiden University,
P.O. Box 9514, 2300RA Leiden, The Netherlands.
Zonneveld@NHN.Leidenuniv.nl

Abstract

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To investigate the origin of cultivated trumpet daffodils the genome size (2C-value) of more than 100 old and new cultivars were measured. A large number of concolored yellow tetraploid cultivars with large coronas seem to be derived from a doubling of chromosomes of a hybrid of the two species *N. hispanicus* Gouan and *N. pseudonarcissus* L. This can also still be recognized by the presence of a black spot at the top of the anthers in about 15 % of the tetraploid cultivars. Assuming *N. pseudonarcissus* as one of the parents of the allotetraploid cultivars, species of trumpet daffodils of section *Pseudonarcissi* investigated earlier were compared. Doubling of the nuclear DNA content of 23.8 pg of *N. pseudonarcissus* falls short of the values found for the trumpet daffodils. Species of trumpet daffodils of section *Pseudonarcissi* with a complementing higher amount of nuclear DNA like *N. hispanicus* with 25.8 pg, *N. poeticus* L. with 26 pg and the hexaploid *N. pseudonarcissus* ssp. *bicolor* (L.) Baker with 67.7 pg (Zonneveld,2008) and other species were evaluated. *N. hispanicus* with 25.8 pg clearly differs from *N. pseudonarcissus* with 23.8 pg and is accepted here also as a species. The nuclear DNA content of *N. bujei* (Fern. Casas) Fern. Casas with 30 pg fits with the (ancient) hybrid origin for *N. bujei* between *N. longispathus* Pugsley (36 pg) and *N. hispanicus* Gouan (25.8 pg). The tetraploids with white tepals and yellow corona's can be obtained by crossing a diploid *N. pseudonarcissus* with the hexaploid and bicolored *N. pseudonarcissus* ssp. *bicolor* (L.) Baker.

Keywords: *Narcissus hispanicus,* daffodil origin, origin of *N. bujei.*

Resumen

Zonneveld, B.J.M. 2010. Participación de Narcissus hispanicus Gouan en el origen de Narcissus bujei y de los narcisos trompeta cultivados (Amaryllidaceae). *Anales Jard. Bot. Madrid* 67(1): 29-39.

Para investigar el origen de los narcisos trompeta cultivados se midió el tamaño del genoma (valor 2C) de más de 100 cultivares viejos y nuevos. Un gran número de cultivares tetraploides amarillos de color uniforme y con grandes coronas parecían derivarse de una duplicación de cromosomas de un híbrido de las dos especies *N. hispanicus* Gouan y *N. pseudonarcissus* L. Esto también se observa por la presencia de una mancha negra en la parte superior de las anteras en alrededor del 15 % de los cultivares tetraploides. Tomando a *N. pseudonarcissus* por uno de los parentales de los cultivares alotetraploides, se compararon especies de narcisos trompeta de la sección *Pseudonarcissi* que ya hubiesen sido investigados anteriormente. La duplicación del contenido de ADN nuclear de 23,8 pg de *N. pseudonarcissus* queda por debajo de los valores hallados en los narcisos trompeta. Se evaluaron otras especies de narciso trompeta de la sección *Pseudonarcissi* con una cantidad más alta de ADN nuclear complementaria, tales como *N. hispanicus,* con 25.8 pg, *N. poeticus* L., con 26 pg y el hexaploide *N. pseudonarcissus* ssp. *bicolor* (L.) Baker, con 67.7 pg (Zonneveld, 2008), entre otras. *N. hispanicus,* con 25.8 pg, se diferencia claramente de *N. pseudonarcissus,* con 23.8 pg, y también se acepta aquí como especie. El contenido de ADN nuclear de *N. bujei* (Fern. Casas) Fern. Casas, con 30 pg, se adecua al hibridismo (antiguo) para *N. bujei* entre *N. longispathus* Pugsley (36 pg) y *N. hispanicus* Gouan (25,8 pg). Los tetraploides con tépalos blancos y coronas amarillas pueden obtenerse con el cruzamiento entre *N. pseudonarcissus* diploide y el hexaploide y bicolor *N. pseudonarcissus* ssp. *bicolor* (L.) Baker.

Palabras clave: *Narcissus hispanicus,* origen de los narcisos trompeta, origen de *N. bujei.*

Introduction

Daffodil culture started with the import of large numbers of bulbs collected from the field in the 16th century. Especially the large concolored yellow *N. hispanicus* (Gouan, 1773) was imported and consequently likely became exterminated in southern France and most of the Pyrenees and later also most of Spain. From these in culture at first triploids originated at the end of the 19th century (Wylie, 1952) with *N.* 'Golden Spur' found in the Estate Bakkershage in Wassenaar, The Netherlands (De Mol, 1923). At roughly the same time the triploids *N.* 'Emperor' and 'Empress' originated with Backhouse UK (Wylie, 1952). At the start of the 20th century the first tetraploids became available (*N.* 'King Alfred' 1899, *N.* 'van Waverens Giant', 1900) and since that time the tetraploid trumpet daffodils have taken over the market.

Previous work in *Hosta* Tratt. (Zonneveld & Van Iren, 2001), *Galanthus* L. (Zonneveld & al., 2003a), *Agapanthus* L'Hér. (Zonneveld & Duncan, 2003b), *Nerine* Herb (Zonneveld & Duncan, 2006), *Narcissus* L. (Zonneveld, 2008) and *Tulipa* L. (Zonneveld, 2009) has shown that nuclear DNA content of species and cultivars can contribute to the taxonomy of species and the origin of hybrids. The *N. pseudonarcissus* L.

Table 1. *Narcissus* species with their amount of nuclear DNA, standard deviation, chromosome number and origin. Chromosome counts are from literature or derived from the genome size.

Coll.#	Species	DNA in pg	Average	St. Dev.	Chrom. #	Origin
PB436	*N. hispanicus* Gouan	25,9	**25,8**	0,3	2*x* = 14*	S. de Nieves, Spain
D031	*N. hispanicus* Gouan	26,6				Cuenca, Spain
BZ 3-'08-1	*N. hispanicus* Gouan	25,5				Los Quejigales, Spain
BZ 3-'08-2	*N. hispanicus* Gouan	25,2				Los Quejigales, Spain
BZ 3-'08-3	*N. hispanicus* Gouan	25,6				Los Quejigales, Spain
BZ 3-'08-4	*N. hispanicus* Gouan	25,7				Los Quejigales, Spain
BZ 3-'08-5	*N. hispanicus* Gouan	25,5				Los Quejigales, Spain
	N. hispanicus Gouan	25,6				ex H. Meeuwissen
BZ 3-'08-6	*N. hispanicus* Gouan	39,3	**39,3**	0,7	2*n* = 3*x* = 21	1 km from Los Quejigales
L16	*N.* 'Hispanicus Maximus'	36,5	**35,4**	0,9	2*n* = 3*x* = 21	H. Bulborum, Limmen
B35	*N.* 'Hispanicus Maximus'	36,1				ex C. Breed
	N. 'Hispanicus Maximus'	34,7				ex H.Meeuwissen
	N. 'Hispanicus Maximus'	35,3				ex C. Breed
	N. 'Hispanicus Maximus'	34,4				ex B. Duncan ex JWB
A8715	*N. bujei* Fern. Casas	29,3	**30,2**	0,5	2*n* = 2*x* = 14	Cabra, Spain
D678	*N. bujei* Fern. Casas	30,5				Albacete, Spain
D701	*N. bujei* Fern. Casas	30,0				F. de la Pieta, Spain
D008	*N. bujei* Fern. Casas	30,1				ex M. Salmon SF137
BZ 3-2008	*N. bujei* Fern. Casas	29,5				10 km w v Cazorla
kw9613	*N. bujei* Fern. Casas	31,5				N. S del Sierra, Spain
	N. pseudonarcissus L.		**23,8**	0,4	2*x* = 14*	Zonneveld (2008)
	N. abscissus (Haw.) Schult. f.		**26,4**	0,8	2*x* = 14&	Zonneveld (2008)
	N. moleroi Fern. Casas		**26,1**	0,4	2*x* = 14&	Zonneveld (2008)
	N. poeticus L.		**26,0**	0,5	2*x* = 14$	Zonneveld (2008)
	N. cyclamineus DC		**26,2**	0,5	2*x* = 14+	Zonneveld (2008)
	N. longispathus Pugsley		**36,0**	0,7	2*x* = 14*	Zonneveld (2008)
	N. nevadensis Pugsley		**38,2**	1,1	2*x* = 14*	Zonneveld (2008)
	N. pseudonarcissus ssp. *bicolor*		**67,7**	2,0	2*n* = 6*x* = 42	Zonneveld (2008)

* Sañudo (1984); + Sañudo (1985); & Monserrat Martí & Vives (1991); $ Mehra & Sachdeva (1976).

complex has given rise in nature to many forms now accommodated in eight subspecies (Zonneveld, 2008). The genome sizes here measured show that *N. hispanicus* Gouan is also of importance in the development of yellow garden daffodils. It is moreover a good species that can still be found in Southern Spain. Moreover *N. bujei* (Fern. Casas) Fern. Casas is likely derived from a cross between *N. hispanicus* and *N. longispathus* Pugsley. The tetraploids with white tepals and yellow corona's can be obtained by crossing the diploid *N. pseudonarcissus* with the hexaploid *N. pseudonarcissus* ssp. *bicolor* (L.) Baker. Lastly, the tetraploid cultivars with pink to orange color in the corona show the influence of *N. poeticus* L.

The taxonomy of all species of *Narcissus* (Amaryllidaceae) has been investigated recently by flow cytometry (Zonneveld, 2008). The somatic nuclear DNA contents (2C) were shown to range from 14 to 38 picogram for the diploids. The total number of *Narcissus* species was determined as 36, nine more than in *Flora Europaea* and they were divided up in two subgenera and eleven sections. Section *Pseudonarcissi* DC was much more heterogeneous in nuclear DNA content than expected. Sixty five accessions of *N. pseudonarcissus* possessed, with 23.8 pg, similar amounts of DNA. However several species from this section were clearly distinctive in nuclear DNA content. It runs from the diploid *N. primigenius* Fern. Casas with 21.7 pg to the also diploid *N. nevadensis* Pugsley with 38.2 pg. Also *N. cyclamineus* DC, *N. abscissus* (Haw.) Schult. f. and *N. moleroi* Fern. Casas are with about 26 pg clearly different from *N. pseudonarcissus.* For the first time, in eleven accessions, hexaploidy was found in *N. pseudonarcissus* ssp. *bicolor*. A new section *Nevadensis* Zonn. with 30-39 pg of nuclear DNA was split off from the section *Pseudonarcissi* with now 21-27 pg.

The aim of the present study was the desire to know whether nuclear DNA value was of use in determining the origin of trumpet daffodils and of the controversial *Narcissus bujei* (Fern. Casas) Fern. Casas. This is of value for the taxonomy of *Narcissus* and also for improving commercial varieties. Moreover, important pharmacological substances like galanthamines are found in daffodils (Codina, 2002) making it worthwhile to be able to discriminate between the species and show the origin of the main groups of cultivars.

Materials and methods

Plant material

Plant material of the species was mainly obtained from the collections of J. Blanchard (UK) and D. Donnison-Morgan (UK). However, most cultivars were obtained from The Netherlands (KAVB Lisse, Hortus Bulborum Limmen, C.P. Breed, C. van der Veek, H. Meeuwissen, W. Lemmers, and S. de Groot) and a few from the UK (J.M. Grimshaw, M. Salmon and I. Young), from France (L. de Jager), from Germany (G. Knoche) and from the USA (N. Wilson and H. Koopowitz). Care was taken to ensure correct identification of all material, relying also on the expert opinions of J. Blanchard, D. Donnison-Morgan and S. de Groot. In most cases fresh leaves were used from the wild or available in the different collections.

Determination of nuclear DNA content

For the isolation of nuclei, about 0.5 cm of a fresh full-grown leaf was chopped together with a piece of *Agave americana* L. 'Aureomarginata' as internal standard. The nuclear DNA content (2C-value) of *A. americana* was measured as 15.9 picogram (pg) per nucleus with human leukocytes (2C = 7 pg, Tiersch & al., 1989) as the standard. The chopping was done with a new razor blade in a Petri dish in 0.25 ml nuclei-isolation buffer, with 0.01 % RNAse added (Zonneveld & Van Iren, 2001b). After adding 1.8 ml propidium iodide solution (50 mg/l in isolation buffer) the suspension with nuclei was filtered through a 30 µm nylon filter. The fluorescence of the nuclei was measured, 30 and 60 min. after addition of propidium iodide, using a PARTEC CA-II flow cytometer. The more DNA is present in a nucleus, the higher is the intensity of the fluorescence. The 2C DNA content of the sample was calculated as the sample peak mean, divided by the *Agave* peak mean, and multiplied with the amount of DNA of the *Agave* standard. From chopping of a leaf piece of 0.5 cm^2 about 50 000 nuclei could be isolated. For each clone, two to six different runs (determinations) with at least 3000-5000 nuclei were measured with two runs from a single nuclear isolation.

Results and discussion

In Table 2 nuclear genome size is presented for more than 60 trumpet daffodils. These were compared with the genome sizes of all species (Zonneveld, 2008, Table 1). It turns out that doubling of the nuclear DNA content of 23.8 pg of *N. pseudonarcissus* falls short of the values found for the trumpet daffodils (Table 3). Morphological similar looking species of trumpet daffodils of section Pseudonarcissi with a complementing higher amount of nuclear DNA like *N. hispanicus* with 25.8 pg, *N. poeticus* with 26 pg and the hexaploid *N. pseudonarcissus* ssp. *bicolor* with 67.7 pg (Zonneveld, 2008) were selected and data on

Table 2. *Narcissus* cultivars with their amount of nuclear DNA, average, standard deviation, chromosome number, origin and remarks. Chromosome counts are from Mol, 1923; Nagao, 1933; Fernandes & Fernandes, 1946; Fernandes & Neves, 1971; Sañudo, 1985, and Brandham & Kirton, 1985.

Coll.#	Colour	White tepals + pink trumpet	DNA in pg	Average	St. dev	Chrom. #	Origin	Parents
2lim1	2WP	*N.* 'Accent'	49,7	49,4	1,1	2*n* = 4*x* = 28	H. Bulborum Limmen, NL	
2lim2	2WP	*N.* 'Algarve'	50,0				H. Bulborum Limmen, NL	
2lim3	2WP	*N.* 'Arctic Char'	49,3				H. Bulborum Limmen,NL	
2lim4	2WP	*N.* 'Eastern Dawn'	48,3				H. Bulborum Limmen, NL	
2lim6	2WP	*N.* 'Rose Noble'	48,8				H. Bulborum Limmen, NL	
2lim7	2WP	*N.* 'Precocious'	50,7				H. Bulborum Limmen, NL	
2lim8	2WP	*N.* 'Truly Royal'	49,8				H. Bulborum Limmen, NL	
2lim9	2WP	*N.* 'Valinor'	50,6				H. Bulborum Limmen, NL	
2lim10	2WP	*N.* 'Violetta'	49,4				H. Bulborum Limmen, NL	
	1WP	*N.* 'Chanson'	47,3				ex C. Breed	
		White tepals + yellow trumpet					H. Bulborum Limmen, NL	
	1WY	*N.* 'W.P. Milner'	23,0	23,5	0,4		ex C. Breed	
	1WY	*N.* 'Princeps'	23,4				ex H.Meeuwissen	
	1WY	*N.* 'Topolino'	23,8			2*x* = 14	ex commerce	
H3	1WY	*N.* 'W.P. Milner'	23,8			2*x* = 14	ex H. Bot. Leiden	
	1WY	*N.* 'Empress'	38,8	38,9	0,5	3*x* = 21+B	H. Bulborum Limmen, NL	pseudon. × bicolor 4*x*
2lim17	1WY	*N.* 'Bizerta'	48,4	49,6	0,9		H. Bulborum Limmen, NL	
2lim13	1WY	*N.* 'Drummerry'	48,9				H. Bulborum Limmen, NL	
2lim16	1WY	*N.* 'Monticello'	49,0				H. Bulborum Limmen, NL	
2lim14	1WY	*N.* 'Bar None'	49,2				H. Bulborum Limmen, NL	
2lim18	1WY	*N.* 'Irish Mist'	49,4				H. Bulborum Limmen, NL	
2lim19	1WY	*N.* 'Wahkeena'	49,5				H. Bulborum Limmen, NL	
2lim15	1WY	*N.* 'Cyros'	49,7				H. Bulborum Limmen, NL	
	2 1WY	*N.* 'Glory of Noordwijk'	49,9				H. Bulborum Limmen, NL	
2lim12	1WY	*N.* 'Breton'	49,9				H. Bulborum Limmen, NL	
2lim20	1WY	*N.* 'Tudor Groove'	50,1				H. Bulborum Limmen, NL	
2lim11	1WY	*N.* 'Bravoure'	51,9				H. Bulborum Limmen, NL	
	1WY	*N.* pseudon. ssp. *leonensis*	64,0	64,0	1,2		Picos de Europa	

Table 2. (Continuación).

Coll.#	Colour	Yellow tepals + yellow trumpet	DNA in pg	Average	St. dev	Chrom. #	Origin	Parents
G15	1YY	*N.* 'Bowles Early Sulphur'	22,6	23,8	0,1	2*x* = 14	ex commerce	
	1 1YY	*N. gayi* (Henon) Pugsley	23,9				H. Bulborum Limmen,NL	
	7 1YY	*N.* 'Golden Spur'	23,8				H. Bulborum Limmen,NL	
	5 1YY	*N.* 'Henry Irving'	24,4				H. Bulborum Limmen,NL	
	3 1YY	*N.* 'Emperor'	37,8	37,8	0,5	3*x* = 21	H. Bulborum Limmen,NL	pseudon × bicolor? (Rix)
	1YY	*N.* 'Golden Harvest'	46,3	48,1	1,1	4*x* = 28	ex C. Breed	G. Spur × K Alfred
	2YY	*N.* 'Saint Keverne'	46,5				ex C. Breed	
	1YY	*N.* 'Dutch master'	46,9				ex C. Breed	
	1YY	*N.* 'King Alfred'	48,9				ex C. Breed	
	1YYd	*N.* 'Golden Ducat'	47,7				ex C. Breed	
	2YY	*N.* 'Carlton'	47,6				ex C. Breed	
	1YY	*N.* 'Meldrum'	48,5				ex C. Breed	
2lim21	1YY	*N.* 'Golden Perfection'	51,6	53,4	2,4		H. Bulborum Limmen,NL	
	1YY	*N.* 'Rijnveld's Early Sensation'	55,1			2*n* = 33	ex C. Breed	
		Other Cultivars						
	3WO	*N.* 'Aflame'	48,8				ex C. Breed	
	4WY	*N.* 'Argent'	24,4				ex C. Breed	
J5	8YY	*N.* 'Avalanche of Gold'	46,6				ex L de Jager	Bill Welch × tazetta
	7YY	*N.* 'Baby Boomer'	31,2				KABV 10-4-08	Avalanche × jonq
	7YY	*N.* × *intermedius* = Baby Boomer?	31,2				KABV 10-4-08	Avalanche × jonq
	7YY	*N.* 'Baby Moon'	33,7				ex commerce	jonq minor × jonq
	7YY	*N.* 'Baby Moon'	67,5				ex commerce	jonq minor × jonq
	3YO	*N.* 'Bath's Flame'	37,2				ex C. Breed	
	4WY	*N.* 'Bridal Crown'	28,2			2*x* = 17	ex commerce	sport of l'Innocence'
L22	1WW	*N.* 'Celilo'	49,0				H. Bulborum Limmen,NL	Petsamo' × 'Beersheba'
	11YOs	*N.* 'Centannees'	49,2				H. Meeuwissen	
	2YO	*N.* 'Croesus'	36,9				ex C. Breed	
H2	7YW	*N.* 'Dickcissel'	41,3			3*x* = 21	ex Hortus Bot. Leiden	Binkie (2*n* = 28) × jonquilla

Table 2. (Continuación).

Coll.#	Colour	Others cultivars	DNA in pg	Average	St. dev	Chrom. #	Origin	Parents
B24	1YYd	*N.* 'Eystettensis'	23,8				double flowers	pseudon(niet × triandrus)
	6YY	*N.* 'Februari Gold'	37,8			3*x* = 21	ex commerce	Golden Spur × cycl.
	11WYs	*N.* 'Frileuse'	36,4			3*x* = 21	ex commerce	split crown
	8WO	*N.* Geranium	27,4				ex C. Breed	
L19	8WO	*N.* 'Glorious'	29,9			2*x* = 17	H. Bulborum Limmen,NL	poeticus × tazetta
2lim23	8WY	*N.* 'Gran Primo Citronieri'	47,0			2*n* = 32	H. Bulborum Limmen,NL	
	5YY	*N.* 'Hawera'	24,3			2*x* = 14	ex commerce	jonquilla × triandrus
	2WY	*N.* 'Ice Follies'	48,8			4*x* = 28	ex commerce	
	4YYd	*N.* 'Inglescombe'	24,9				ex C. Breed	
	4WOd	*N.* 'Insulinde'	37,2				ex C. Breed	
	6WY	*N.* 'Jack Snipe'	39,0			3*x* = 21	ex commerce	x cyclamineus
	6YO	*N.* 'Jetfire'	37,4			3*x* = 21	ex commerce	x cyclamineus
L18		*N.* 'Jumage'	72,4				H. Bulborum Limmen,NL	x tazetta
	2YO	*N.* 'Killigrew'	48,9				ex C. Breed	
2lim5	2WO	*N.* 'Kilworth'	49,9				H. Bulborum Limmen,NL	
	7YY	*N.* 'Kokopelli'	41,7				ex H.Meeuwissen	Sundial × fern.
L20	7YO	*N.* 'Lanarth'	41,0			3*x* = 21	H. Bulborum Limmen,NL	x jonquilla
L09	8WY	*N.* 'Laurens Koster'	28,3			2*x* = 17	H. Bulborum Limmen,NL	poeticus × tazetta
B5	6YY	*N.* 'Minicycla'	23,8				ex C. Breed	cyclamineus × astur.
H5	8WY	*N.* 'Minnow'	28,0				ex Hortus Bot. leiden	x tazetta
L05	13yy	*N.* 'Mite'	24,5				H. Bulborum Limmen,NL	obvallaris × cyclam.
J4		*N.* 'Monarch × Northland'	49,6				ex L. de Jager	
H13	1WW	*N.* 'Mount Hood'	49,3			4*x* = 28	ex Hortus Bot. Leiden	
	4WO	*N.* 'Orange Phoenix'	24,8				ex C. Breed	
	11YYs	*N.* 'Pampaluna'	48,5				ex C. Breed	y,y split
	6YY	*N.* 'Peeping Tom'	37,1			3*x* = 21	ex commerce	cycl × pseudonarc. 4x
	7YWYW	*N.* 'Pipit'	42,8				ex commerce	Binkie × jonquilla
	5YY	*N.* 'Queen of Spain'	33,5				ex C. Breed	
	3WY	*N.* 'Queen of the North'	36,1				ex C. Breed	
	4YYd	*N.* 'Rip van Winkel'	22,8				ex H.Meeuwissen	
	2WO	*N.* 'Royal Orange'	49,6				H. Meeuwissen	

Table 2. (Continuación).

Coll.#	Colour	Others cultivars	DNA in pg	Average	St. dev	Chrom. #	Origin	Parents
BZ	3WY	*N.* 'Segovia'	26,9				ex commerce	rup watieri? × ?
	2YY	*N.* 'Sir Watkin'	36,3				ex C. Breed	
		N. 'Snow Tube' ?	48,2				KABV	
H14	7YY	*N.* 'Sun Disc'	25,9			2*x* = 14	ex Hortus Bot. Leiden	rupicola × (poeticus?)
	7YY	*N.* 'Sundial'	26,2				ex C. Breed	poeticus × rupicola
	2WY	*N.* 'Sweet Harmony'	50,2				H. Meeuwissen	
G16	YYd	*N.* 'Telemonius Plenus'	24,3			2*x* = 14	ex J. Grimshaw	= 'Van Sion'
	12YY	*N.* 'Tete-a-Tete'	39,7			3*x* = 24	ex commerce	(CyclxSoleildÖr)nonredOP
	3WO	*N.* 'The Star'	25,2				ex C. Breed	
	3YO	*N.* 'Twinkle'	36,4				ex C. Breed	
	1YYd	*N.* 'Wavertree'= 'Giant'	23,3				ex C. Breed	coll. asturiensis
	2WO	*N.* 'Will Scarlet'	26,2				ex C van der Veek	absc xpoet poetarum
	YY	N. × jonquilla	34,9				KABV	
	3WW	N. 'Xit'	25,6				ex C. Breed	
B34	4YYd	N. 'Yellow Cheerfulness'	40,9			3*x* = 24	ex C. Breed	Elvira' ->'Y. Cheerfulness'

these and other related species are here presented (Table 1). Species of other sections can be excluded as these have mainly short trumpets and/or result (with exception of *N. poeticus)* in sterile offspring. For comparison the results for 61 other cultivars are also given.

The establishment of N. hispanicus *as a species*

In 2008 I found a *Narcissus* in fair numbers in the Parc Natural Los Quejigales in the Sierra de las Nieves amidst *Abies pinsapo* Boiss. forests that turned out to have a 2C-value of 25.8 pg. Other accessions from the same area have similar 2C-values (Table 1). An exception was a plant found 10 km west of Los Quejigales that with 39.3 pg clearly is a triploid. Meadows (1972) found *N. hispanicus* in a remote just opened spot in the Pyrenees, Val Cardos, Pleta de Tornapigol, but this was not available for study. This amount of on average 25.8 pg of nuclear DNA is clearly different from the 23.8 pg for *N. pseudonarcissus*. The plants from Los Quejigales conform with *N. hispanicus* as described by Gouan (1773). It is characterized by big bright yellow colored flowers with spiraled tepals, an ascending posture and a minute black spot on the anthers. In 1788 Curtis described the same species as *N. major*. Baker (1888) included it as a subspecies of *N. pseudonarcissus*. The term 'Major' or 'Maximus' was later used for (m)any large yellow daffodils. *N.* 'Hispanicus Maximus' in culture turns out to be a triploid (Table 1). *Narcissus hispanicus* 'Pinetorum' PB436 with 25.8 pg was found by P. Bird in Sierra de las Nieves and via M. Salmon grown by J. de Groot from seed. It is presumably the same plant as described by Smythies (1973) from the Sierra de las Nieves in southern Spain as growing amidst *Abies pinsapo.* According to Smythies (1973) Willkomm collected this species in 1861 in the Sierra de Yunquera (now Sierra de las Nieves) at 1350 m and considered them to be *N. major*. Webb (1980) considered *N. hispanicus* as a form of *N. pseudonarcissus* ssp. *major*. However Pugsley (1933), Fernandes (1968) and Fernandes Casas (1986) regarded *N. hispanicus* as a good species. Moreover, Graham & Barrett (2004) could separate *N. hispanicus* from *N. pseudonarcissus* in their phylogeny of *Narcissus* based on DNA sequences of two chloroplast genes. The arguments presented above supports species rank of *N. hispanicus*. Its origin seems to be the Pyrenees, Southern France (rare nowadays in both cases) and the Sierra de las Nieves in southern Spain.

Species incorrectly thought to be N. hispanicus

Plants from Zezere Portugal described by Fernandes as *N. hispanicus, N. confusus* Pugsley from Sie-

rra de Gredos, and from Embalse de Abraham, Sierra Higuera, described by Fernández Casas as allied to *N. obvallaris* (Henon) Pugsley are just plain *N. pseudonarcissus* with 23.8 pg (Zonneveld, 2008). *Narcissus obvallaris* Salisb. and *N. gayi* Salisb., both of doubtful origin, have an amount of nuclear DNA similar to *N. pseudonarcissus*. However they have a black spot on the anthers (Blanchard 1990; 1998). So it could be suggested that occasionally *N. pseudonarcissus* has a black spot. More likely, they show the influence of *N. hispanicus*, although their amount of DNA does not reflect that anymore.

The origin of N. bujei *(Fern. Casas) Fern. Casas*

Section *Nevadensis* Zonn. was separated from section *Pseudonarcissi* based on its much higher amount of DNA of 36-38 pg (Zonneveld, 2008). At first, based on 50% more DNA, they seemed triploids of *N. pseudonarcissus*. However, they have as far as they were counted $2n = 14$ chromosomes (Sanudo, 1984) and fully fertile pollen. So *N. nevadensis* Pugsley (1933) with 38 pg and *N. longispathus* and its three subspecies (Rois-Ruiz & al., 1998; Zonneveld, 2008) with 36 pg are clearly separated from *N. pseudonarcissus*.

The *N. bujei* with 30.2 pg is described from the Serrania de Ronda, southern Spain. It is clearly separate in nuclear DNA content but hardly morphologically from both *N. pseudonarcissus* and *N. longispathus* or *N. nevadensis*. However, the latter two are characterized by having usually more than one flower to a stem. *Narcissus bujei* was first described as a variety of *N. longispathus (N. longispathus* var. *bujei* Fern. Casas: "amicissimo Eugenio Domínguez Vilches, familiariter nobis 'Buje', ex animo dicata", the orthography therefore is with an "j", not a "g"), later as a variety of *N. hispanicus* and then as a species all by the same author (Fernández Casas, 1986, "*bugei*"), who in 2000 changed his mind again and made it a subspecies of *N. hispanicus*. Both *N. bujei* and *N. hispanicus* are described as having bright yellow flowers, with spirally twisted tepals and leaves and a black spot on the anthers. These data fit with the (ancient) hybrid origin for *N. bujei* (30.2 pg) between *N. longispathus* (36 pg) and *N. hispanicus* (25.8 pg).

Table 3. Calculated genome sizes of *Narcissus* species, hybrids and cultivars compared with the actual measurements.

Diploids	Genome size (2C) calculated, in pg
N. pseudonarcissus (measured)	23,8
N. hispanicus (measured)	25,8
N. pseudo × hisp	24,8
Triploids	
N. pseudo × pseudo × pseudo	35,7
N. (pseudo × hisp) × pseudo	36,7
N. (pseudo × hisp) × hisp	37,7
N. hisp × hisp × hisp	38,7
Tetraploids	
N. pseudo × pseudo × pseudo × pseudo	47,6
N. pseudo × pseudo × pseudo × hisp	48,6
N. pseudo × hisp × pseudo × hisp	49,6
N. pseudo × hisp × hisp × hisp	50,6
N. hisp × hisp × hisp × hisp	51,6
	Measured
N. hispanicus 'Maximus'.Hort.	36,3
N. 'Emperor'	37,8
N. 'Empress'	38,9
N. 'King Alfred'	49,0
N. Yellow, Yellow Corona (Table 2)	48,1
N. White, Yellow Corona (Table 2)	49,6
N. White, Pink Corona (Table 2)	49,4

The origin of cultivated trumpet daffodils

In the most recent taxonomical treatment of *Narcissus* (Zonneveld, 2008) seven species are attributed to the section *Pseudonarcissi* DC. with a 2C-value of about 23.8 pg for all subspecies of *N. pseudonarcissus*. The calculations in Table 3 show that plain tetraploids from *N. pseusonarcissus* (23.8 pg) will have a 2C-value of 47.6 pg. The nuclear DNA content of the large trumpet daffodils have higher values (Table 2). This suggests that a species of trumpet daffodil with a higher amount of nuclear DNA is also involved in the formation of the trumpet daffodils that are grown nowadays. Three morphological similar species of trumpet daffodils of section Pseudonarcissi have a 2C-value of about 26 pg: *N. abscissus* Schult. f., *N. moleroi* Fern. Casas and *N. hispanicus* Gouan. *N. abscissus* has a cut-off corona, a character rarely encountered in today's daffodils, whereas the rare *N. moleroi* has pale colored flowers. So both seem hardly involved in today's breeding. Although *N. cyclamineus* DC. and *N. poeticus* L. from section *Narcissus* (Zonneveld, 2008) have a similar genome size of about 26 pg, they differ too much in flower morphology to con-

sider them as being involved with the origin of the yellow trumpet daffodils. This leaves us with *N. hispanicus*. *N. hispanicus* is characterized by big bright yellow colored flowers with spiraled tepals, an ascending posture and minute black spots on the anthers. Apart from the spiraled tepals these characters can be found back in today's cultivars. A plain tetraploid *N. hispanicus* (25.8 pg) will have a 2C-value of 51.6 pg, higher than any of the cultivated trumpet daffodils. This suggests that both *N. hispanicus* and *N. pseudonarcissus* are involved. The diploid plant from Sierra de las Nieves has a 2C-value of about 26 pg. The following can be envisioned: this mountain range is one of the few remaining places were *N. hispanicus* survived. *N. hispanicus* has been imported repeatedly in the old days. This might be due to the fact that it is not very well adapted to culture in N. Europe and it might resent the summer wetness and winter frosts. Despite that and also despite its spiraled tepals, *N. hispanicus* has several characters worthwhile to introduce. First it has big bright yellow colored flowers, whereas those of *N. pseudonarcissus* are usually whitish to pale yellow. Further, contrary to *N. pseudonarcissus* that usually has drooping flowers, the flowers have an ascending posture. The main character contributed by *N. pseudonarcissus* maybe its hardiness and non-spiraled tepals. The hybrid seedlings with spiraled tepals where said to have "mill wings" and vehemently selected against (W. Lemmers, pers. comm.). Moreover the hybrid might have shown hybrid vigour. The diploid plant here described under *N. hispanicus* has been crossed in culture with *N. pseudonarcissu*s. The hybrid might have been partly fertile and, as is often the case with partly sterile hybrids, might have formed non-reduced ovules. These have given rise to the triploid *N.* 'Hispanicus Maximus'. The plant in culture as *N. "hispanicus"* turns out to be this triploid. The 2C-value of 36.3 pg leads to envision a non-reduced diploid gamete of *N. pseudonarcissus* × *N. hispanicus* with a haploid gamete of *N. pseudonarcissus* or *N. hispanicus*. Around 1860 three English breeders found triploid daffodils. They were bought by Barr who brought them in circulation in 1875 (Wylie, 1952). The varieties *N.* 'Emperor' and *N.* 'Empress' were also triploid. In 1890 tetraploids arose from them like *N.* 'King Alfred', that is supposed to be a hybrid of *N.* 'Empress' with *N. hispanicus* (Williams, 1929), and in The Netherlands the triploid *N.* 'Golden Spur' was followed by the tetraploid *N.* 'van Waverens Giant'. However, the plant in culture as *N.* 'Golden Spur' turned out to be diploid (Table 2). In these cases the involvement of *N. hispanicus* was already suggested at the time when they appeared on the market.

The cultivars mainly from Hortus Bulborum, Limmen, The Netherlands with 48.1 pg and with yellow tepals and trumpets most conveniently can be explained as being allotetraploids of *N. pseudonarcissus* and *N. hispanicus*. Autotetraploid *N. pseudonarcissus* would have a 2C-value of 47.6 pg and autotetraploid *N. hispanicus* would have a 2C-value of 51.6 pg. Counts of Melissa Reading and Boyce Tankersley in the USA and myself in the Hortus Bulborum of Limmen (NL) have shown that up to 15 % of the narcissi with large trumpets (division 1 and 2) have black spots at the tip of the anthers. These black spots, the bright yellow colors and upright posture shows the influence of *N. hispanicus* in today's daffodil breeding.

Daffodils with white tepals and with yellow trumpets

These have a 2C-value of 49.6 pg (Table 2) and have likely been derived from crosses between hexaploid *N. pseudonarcissus* ssp. *bicolor* from the Pyrenees and Southern France and/or ssp. *leonensis* (Pugsley) Ferd. Casas & Laínz from Northern Spain and a diploid species, giving a tetraploid in the first generation. Both wild hexaploid daffodils have white tepals and large yellow trumpets.

Daffodils with pink or orange to red corona's

All tetraploid cultivars around with orange to pink corona's are derived from *N. poeticus* and especially from the tetraploid form *N. poeticus* 'Actaea'. Tetraploids with white tepals and pink trumpets with large corona's have on average a 2C-value of 49.4 pg. These are clearly derived from *N. poeticus* with a 2C-value of 26 pg or its tetraploid cultivar 'Actaea' with 52 pg and *N. pseudonarcissus*. Fernandes (1968a; 1968b) remarks on the similarity of the karyotypes of *N. poeticus* of section Narcissus and section *Pseudonarcissi*. As the hybrid between these two seems to be the only fertile intersectional hybrid one may wonder whether or not these two sections should be united. This is also indicated by the results of Graham & Barrett (2006). The red or pink color in today's daffodil corona's that is exclusively derived from *N. poeticus* and the black spot on the anthers are both unique characters. However in the first case there was and still is a very strong selection for the red color. It is very unlikely anyone has given any attention to the minute black spot on the anthers. The black spots are absent from the investigated cultivars that show the red color of *N. poeticus* or the white tepals and yellow corona's derived from *N. pseudonarcissus* ssp. *bicolor*.

In Table 3 2C-values of different combinations of species are calculated. If these are compared with the actual data found (Tables 1, 3) and also taking the flower colors into account, it is clear that *N. hispanicus* played a role in the tetraploid yellow cultivars, *N. poeticus* in the cultivars with red or pink corona's and *N. pseudonarcissus* ssp. *bicolor* in the tetraploids with white tepals and yellow corona's.

This is the first time that the difference in nuclear DNA content is shown between *N. hispanicus* and *N. pseudonarcissus* and is used to explain the origin of *N. bujei* and the yellow tetraploid cultivars. Also the DNA hexaploidy of *N. pseudonarcissus* ssp. *bicolor* and its use in obtaining tetraploid bicolored trumpet daffodils was not recorded earlier. These data allowed giving a firm footing to the supposed involvement of these species in the origin of the trumpet daffodils.

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