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Pollen Volume and Chromosome Content of Daffodils; Possibilities for hybridizing 2

Since 1996 I make crosses between standard daffodils and species. The standard daffodils are tetraploid,that means they have four chromosome sets. The species in most cases are diploid with two chromosome sets. The descendants possess therefore two sets of the standard daffodils and one set of the species and should be infertile because of this configuration. For ideal fertility it must be possible to divide the number of different chromosome sets of the plant by two and get complete sets for the egg cell and the pollen.

In the last three years, I found out that some of the seedlings which should be infertile show a little number of sprouting pollen (1). But what about the chromosome content of these pollen? If they have the right content they can be of great value for further crosses. It should be very complicated if not impossible at this time to determine the chromosomes of the pollen by direct observation. But perhaps the pollen volume can give some hints.

For many plants as for example roses, Achillea borealis, Hordeum, Ipomea and clover was found out that pollen grains from tetraploid plants with two times the chromosome content of diploid plants show a volume which is nearly doubled. The pollen grains of daffodils have a form which is roughly an ellipsoid with the minimum diameter ‘a’ and the maximum diameter ‘b’ . On this basis the volumes within Tables 1 to 6 were calculated. In Table 1 you find for Actaea a volume of 57,6 µmm³ (Picture 1). This tetraploid garden daffodil came into being from the diploid Narcissus poeticus with a volume of 29,7 µmm³(Picture 2). For this example the presumption that the volume of the pollen grain becomes doubled with the doubling of the chromosome content is nearly perfect. But why is the mean value for the tested garden daffodils 71,8 µmm³? This could be the influence of some pseudonarcissus species which took part together with N. poeticus in the development of the tetraploid garden daffodils.

Picture 1 Pollen of Actaea

Picture 2 Pollen of N. poeticus

Table 1 Pollen of tetraploid garden daffodils

Varity	Z	a/b in µm	V ± SD in µmm³	chromosome set/ pollen	chromosome set/ plant
TS 290	3	46/51	55,3 ±13,6	NN	NNNN
Lady Eve	2	50/54	70,9 ± 2,6	NN	NNNN
Maria Pia	3	42/47	46,0 ± 7,3	NN	NNNN
Brooke Ager	3	51/58	79,2 ± 5,0	NN	NNNN
Ballygowan	4	51/67	90,0 ± 5,9	NN	NNNN
Loch Coire	4	57/61	101,6 ±28,0	NN	NNNN
Actaea	11	44/57	57,6 ± 5,6	NN	NNNN
Florida Manor	7	50/62	82,4 ± 20,9	NN	NNNN
TS 950	12	44/56	59,1 ±10,6	NN	NNNN
TS 1000	11	50/60	77,7 ± 9,9	NN	NNNN
TS 162	4	51/62	83,8 ± 2,6	NN	NNNN
TS 313	2	49/67	82,7 ± 11,5	NN	NNNN
Catalyst	2	42/54	46,9 ± 0,3	NN	NNNN
Upalong	3	42/58	53,9 ± 2,3	NN?	NNNN?
UFO	10	48/59	70,5 ± 10,6	NN	NNNN
Hyperbole	5	54/61	93,4 ± 10,7	NN	NNNN
Corofin	7	43/58	57,5 ± 11,0	NN	NNNN
Neon Blaze	4	47/62	71,7 ± 7,1	NN	NNNN
Elfin Dell	4	43/54	50,7 ± 1,5	NN	NNNN

Z= number of measured pollen grains, a= mean minimum pollen grain diameter, b= mean maximum pollen grain diameter, V = mean pollen volume, SD = standard deviation

Pollen of diploid species normally must be haploid, but sometimes the cell division during reduction division doesn't take place and the pollen is diploid (unreduced). In these cases the pollen volume should be doubled. This is so with good approximation for some pollen of N. fernandesii and N. assoanus 2 in Table 2. Concerning to this behaviour, each species and each clone of a species are different. Some have no unreduced pollen and some have 30 % as the N. assoanus 2 clone.

A more far-reaching assumption is that the pollen volume of daffodils which have different chromosome sets can be calculated by the addition of the volume parts of these chromosome sets. If this is made for the pollen of jonquilla hybrids (Table 3) and viridiflorus hybrids (Table 4) with the mean values of each group the results are relatively good. These mean values would be more reliable if in each group more varieties would have been examined, especially to get a similar effect of poeticus and pseudonarcissus genes of the garden daffodils. The deviation within the specific

Table 2 Pollen of species

Species	**Z**	**a/b in µm**	**V ± SD in µmm³**	**CPO**	**CPL**	**Site**
N. tazetta	11	31/40	19,3 ± 2,1	T	TT	Figueres
N. elegans	9	29/35	14,4 ± 2,3	E	EE	Antequera
N. viridiflorus	5	31/49	25,2 ± 3,9	VV	VVVV	Conil
N. fernandesii	8	26/42	15,7 ± 3,1	F	FF	*Sierra Madrona*
	1	33/56	32,3	FF		
N. cordubensis	7	26/37	14,4 ± 2,1	J	J	Grazalema
N. cuatrecasasii	4	26/40	14,9 ± 1,8	Cu	CuCu	Grazalema
N. assoanus 1	5	28/39	15,2 ± 2,3	A	AA	Alcossebre
N. assoanus 2	12	26/36	12,6 ± 3,9	A	AA	Villarluengo
	8	33/46	27,0 ± 3,9	AA		
N. gaditanus	4	29/43	18,3 ± 2,0	G	GG	Las Negras
N. scaberulus	4	24/35	10,2 ± 1,3	Sc	ScSc	Bobadela
N. calcicola	3	26/40	14,2 ± 1,3	Cl	ClCl	Minde
N. watieri	3	22/37	10,2 ± 0,3	W	W	High Atlas
N. triandrus pallidulus	8	29/41	17,9 ± 3,3	Tp	TpTp	Santa Elena
N. triandrus concolor	7	29/43	18,9 ± 2,3	Tc	TcTc	Monte Gordo
N. cyclamineus	4	31/49	24,8 ± 5,8	Cy	CyCy	Santiago
N. asturiensis	3	29/43	18,5 ± 3,0	As	AsAs	Serra da Estrela
N. poeticus	4	34/50	29,7 ± 1,2	Po	PoPo	Bourisp
N. cavanillesii	8	28/42	17,0 ± 1,2	Cv	CvCv	Conil
N. miniatus	4	35/42	25,5 ± 3,4	ESe	EESeSe	Conil
N. dubius	9	31/42	21,7 ± 2,9	AAP	AAAAPP	Santa Eulalia
N. tortifolius	7	31/43	22,1 ± 2,3	AP	AAPP	Turre
N. bulbocodium 1	4	38/60	45,5 ± 2,2	B	BB	Sierra Madrona
N. bulbocodium 2	7	42/71	64,9 ± 5,0	BB?	BBBB?	Santiago
N. bulbocodium 3	2	42/61	55,5 ± 3,6	BB?	BBBB?	Sabres/Landes
N. hedraeanthus	5	41/62	54,3 ± 11,2	H	HH	Santa Elena
N. cantabricus	5	37/68	48,6 ± 8,1	C	CC	Calatrava
N. cantabricus foliosus	4	42/67	63,2 ± 7,2	CC	CCCC	Morocco
N. romieuxii	3	39/67	53,2 ± 10,2	RR	RRRR	Morocco
N. hispanicus	8	36/44	29,9 ± 5,1	Hi	HiHi	S. de Nieves

Z = number of measured pollen grains, a= mean minimum pollen grain diameter, b= mean maximum pollen grain diameter, V = mean pollen volume, SD = standard deviation, CPO = chromosome sets of pollen, CPL = chromosome sets of the plant, Se = chromosome set of N. serotinus, P = chromosome set of N. papyraceus

groups is big because of the influence of the special garden daffodil. The influence of the species should be constant. This calculation can also be made for the fertile allotetraploid tazetta hybrid Matador and the fertile allotetraploid triandrus hybrid Lapwing. For the two chromosome sets of the pollen of Lapwing (Table 6) one from garden daffodils with a mean value of 35,9 µmm³ and one from N.triandrus with a value of 17,9 µmm³ the sum is 53,8 µmm³. The measured value is 50,2 µmm³. In many cases the accordance is not so good. It should be better when the calculation is based on the garden daffodil parent of the special cross. But also this is far from the ideal situation, because the garden daffodil can prefer genes for the pollen size from a grandparent.

The pollen volume of species (Table 2) is with the exception of the bulbocodiums much smaller than that of the groups above. The mean value for diploid species without the bulbocodiums is 17,1.µmm³. Some species as N. viridiflorus, N. tortifolius

,N. dubius, N. cantabricus foliosus and N. romieuxii are tetraploid or hexaploid. It is to be seen that the principle for calculating the pollen volume as above cannot be used. The pollen grains of N. viridiflorus are a little smaller than they should be, of N. tortifolius about one third, of N. dubius the half. The same is right for N. cantabricus foliosus; the pollen volume should be much greater. Here perhaps the question is: Which pollen volume is the best in the course of evolution? This volume

Table 3 Pollen of jonquilla hybrids

Variety	Z	a/b in µm	V ± SD in µmm³	chromosome set/ pollen	chromosome set/ plant
Quick Step	6	46/54	58,6 ± 10,3	NJ	NNJJ
Regeneration	5	53/57	82,7 ± 7,0	NJ	NNJJ
Pink Step	9	36/51	36,6 ± 7,8	NJ	NNJJ
Jonquillan	4	42/53	46,7 ± 9,5	NJ	NNJJ
	2	64/74	157,2 ± 12,6	NNJJ	
TS 411	9	42/54	48,6 ± 7,2	NJ	NNJJ
TS 412	7	46/60	65,1 ± 9,1	NJ	NNJJ
TS 413	4	44/56	54,8 ± 5,8	NJ	NNJJ
TS 414	7	42/54	49,2 ± 11,2	NJ	NNJJ
Limequilla	14	47/61	69,3 ± 7,1	NJ	NNJJ
NNJJ Brian	8	45/57	60,3 ± 11,8	NJ	NNJJ
Artist's Life	9	41/56	48,6 ± 8,4	NJ	NNJJ

Z = number of measured pollen grains, a = mean minimum pollen grain diameter, b = mean maximum pollen grain diameter, V = mean pollen volume, SD = standard deviation

Table 4 Pollen of viridiflorus hybrids

Variety	Z	a/b in µm	V ± SD in µmm³	CPO	CPL
Emerald Sea	4	43/56	54,3 ± 4,2	NV	NNVV
TS 108 x N. viridiflorus 1	6	35/51	34,0 ± 1,8	NV	NNVV
TS 108 x N. viridiflorus 2	5	36/56	37,7 ± 1,8	NV	NNVV
TS 108 x N. viridiflorus 3	6	38/56	43,3 ± 1,7	NV	NNVV
TS 108 x N. viridiflorus 4	5	36/48	33,5 ± 3,3	NV	NNVV
Corofin x N. viridiflorus 1	9	37/49	34,7 ± 6,1	NV	NNVV
Corofin x N. viridiflorus 2	5	36/51	35,4 ± 3,2	NV	NNVV
Ballygowan x N. viridiflorus	9	40/54	46,9 ± 7,1	NV	NNVV
Fragrant Rose x N. viridiflorus	11	44/56	58,3 ± 8,7	NV	NNVV
Reference Point x N. viridiflorus	10	40/53	43,8 ± 4,9	NV	NNVV
Actaea x N. viridiflorus	10	37/52	36,8 ± 5,0	NV	NNVV
TS 125 x N. viridiflorus	14	42/54	48,8 ± 9,1	NV	NNVV
	6	49/66	83,8 ± 14,4	NNVV	

Z = number of measured pollen grains, a = mean minimum pollen grain diameter, b = mean maximum pollen grain diameter, V = mean pollen volume, SD = standard deviation, CPO = chromosome sets of pollen, CPL = chromosome sets of the plant

will be reached after very many generations for a definite species. A short time after the emergence of a new tetraploid or hexaploid daffodil the pollen volume is a function of the chromosome content, but not for very long times. Here the ecological factors of the site are of effect, as for example the insects which take the pollen grain from one flower to the other.

A good correlation between pollen volume and the nuclear DNA content of the plant cell determined by B.J.M. Zonneveld (2) doesn't exist for species. His values describe the tetraploid, allotetraploid and hexaploid species in relation to diploid plant cells exactly.

Another question is: How much is the pollen size influenced by the conditions for growing and different clones of species? I think the force is small if you don't have extreme growth conditions. The effect for different clones of N. elegans, N. viridiflorus, N. miniatus, N. cantabricus and N. cyclamineus is not too big (Table 5).

In Table 6 you find crosses which should be infertile because of the irregular chromosome content of the plant cell. It is to be seen that for the listed plants some fertility exists. Most crosses are of the type NNX which are generated by combining

Table 5 Pollen of clones

Species	Z	a/b in µm	V ± SD in µmm³	CPO	CPL	Site
N. elegans 1	9	29/35	14,4 ± 2,3	E	EE	Antequera
N. elegans 2	9	28/35	14,1 ± 1,1	E	EE	„
N. elegans 3	9	28/37	15,8 ± 1,3	E	EE	„
N. viridiflorus 1	6	31/42	20,9 ± 4,4	VV	VVVV	Conil
N. viridiflorus 2	5	31/49	25,2 ± 3,9	VV	VVVV	„
N. viridiflorus 3	4	32/47	25,3 ± 3,9	VV	VVVV	„
N. miniatus 1	4	35/42	25,5 ± 3,4	ESe	EESeSe	Conil
N. miniatus 2	10	32/43	22,8 ± 3,4	ESe	EESeSe	„
N. cantabricus 1	5	37/68	48,6 ± 8,1	C	CC	Calatrava
N. cantabricus 2	5	39/55	44,6 ± 9,8	C	CC	„
N. cantabricus 3	5	39/68	53,8 ± 6,8	C	CC	„
N. cyclamineus 1	4	30/40	18,9 ± 1,6	Cy	CyCy	Santiago
N. cyclamineus 2	4	31/49	24,8 ± 5,8	Cy	CyCy	„
N. cyclamineus 3	6	27/44	17,4 ± 4,0	Cy	CyCy	„

Z = number of measured pollen grains, a = mean minimum pollen grain diameter, b = mean maximum pollen grain diameter, V = mean pollen volume, SD = standard deviation, CPO = chromosome sets of pollen, CPL = chromosome sets of the plant

Table 6 Pollen of different crosses

Variety Bellsong	Z	a/b in µm	V ± SD in µmm³	N	CPO	CPL
Bellsong	5	47/58	66,4 ± 5,2	30	NJ	NNJ
	5	53/59	87,3 ± 6,1		NNJ	
Altruist x N. cordubensis 1	9	43/56	55,2 ± 8,1	30	NJ	NNJ
Altruist x N. cordubensis 2	10	42/52	49,5 ± 5,9	73	NJ	NNJ
Altruist x N. cordubensis 3	3	43/49	50,5 ± 9,6	7	NJ	NNJ
2Y-R x N. cordubensis	4	56/69	112,2± 3,7	9	NNJ	NNJ
Trigonometrie x N. cordubensis	8	42,53	51,4 ± 13,4	55	NJ	NNJ
Altruist x N. fernandesii 1	5	43/54	53,7 ± 8,2	13	NF	NNF
Altruist x N. fernandesii 2	7	52/57	79,5 ± 12,2	27	NNF	NNF
	2	35/47	30,0 ± 5,8		N	
Altruist x N. fernandesii 3	1	44/61	63,2	14	NF	NNF
	2	34/52	31,7 ± 4,8		N	
TS 35 x N. fernandesii	6	39/53	43,8 ± 8,0	17	NF	NNF
Penvale	2	43/53	51,3 ± 4,7	21	NJ	NNJ
	2	50/67	87,2 ± 0		NNJ	
TS 108 x N. cuatrecasasii 1	1	42/56	50,5	58	NCu	NNCu
TS 108 x N. cuatrecasasii 2	2	49/54	67,0 ± 2,4	8	NNCu	NNCu
Largo x N. cuatrecasasii	1	42/53	47,7	10	NCu	NNCu

	1	53/56	81		NNCu	
Pinza x N. cuatrecasasii	7	43/61	61,0± 14,1	21	NCu	NNCu
TS 125 x N. assoanus	3	37/49	34,3 ± 0,4	19	N	NNA
	2	45/56	59,2 ± 2,6		NA	
Matador	3	48/56	67,5 ± 4,5	62	NT	NNTT
Actaea x N. tazetta 1	2	31/42	20,4 ± 0	13	N or T	NNT
Actaea x N. tazetta 2	7	35,47	29,4 ±4,0	60	N	NNT
	2	39/50	40,3 ± 5,1		NT	
Actaea x N. tazetta 3	7	42/49	45,8 ± 8,7	25	NT	NNT
Actaea x N. tazetta 4	3	38/49	38,0 ± 2,8	19	NT	NNT
Actaea x N. tazetta 5	3	39/49	38,7 ± 0,9	13	NT	NNT
	4	34/48	28,5 ± 3,5		N	
Actaea x N. tazetta 6	5	36/43	30,0 ± 6,1	29	N	NNT
Actaea x N. tazetta 7	4	42/51	46,6 ± 9,0	98	NT	NNT
Actaea x N. tazetta 8	2	37/49	35,9 ± 5,2	30	NT	NNT
Actaea x N. tazetta 9	2	37/43	30,5 ± 0,2	15	N	NNT
Loch Coire x N. tazetta 1	7	50/59	77,5 ± 14,7	14	NT	NNT
Loch Coire x N. tazetta 2	3	47/55	63,0 ± 14,7	8	NT	NNT
Loch Coire x N. tazetta 3	4	56/59	99,4 ± 10,2	15	NNT	NNT
Loch Coire x N. tazetta 4	5	68/83	203,1 ± 32,9	18	2 NNT	NNT
	1	50/58	76,3		NT	
Ufo x N. tazetta	1	56/56	89,7	5	NNT	NNT
Geranium	3	51/51	69,3 ± 6,6	135	NT	NT
	3	58/62	110,6 ± 10,5		2NT	
Pontresina x N. pannazianus 1	6	48/53	65,5 ± 13,5	49	NP	NNP
Pontresina x N. pannazianus 2	9	48/54	67,8 ± 19,2	32	NP	NNP
Grand Monarch	4	58/65	118,1 ± 31,7	65	2TTP	TTP
	5	*37/45*	*32,9 ± 5,6*		TP	
Lapwing	3	43/50	50,2 ± 7,2	21	NTr	NNTrTr
Chipper	10	45/51	54,8 ± 10,7	10	NTr	NNTr
Loch Loyal x N. triandrus pallidulus	3	42/46	42,4 ± 8,4	4	NTp	NNTp
Wychavon x N. triandrus pallidulus	1	50/53	69,0	6	NTp	NNTp
TS 2 x N. triandrus pallidulus	4	57/62	105,4 ± 16,8	5	NNTp	NNTp
TS 2 x N. triandrus pallidulus	3	68/69	170,7 ± 7,9	10	2NNTp	NNTp
TS 2 x N. triandrus pallidulus	2	48/56	66,8 ± 2,7	37	NTp	NNTp
Ufo x N. cantabricus	8	60/70	142,5 ± 17,3	25	NNC	NNC
	3	50/56	71,9 ± 15,6		NC	
Honeybird x N. hedraeanthus	4	55/73	113,4 ± 7,9	23	NNH	NNH
	2	47/57	64,6 ± 5,0		NN	
Articulate	4	40/56	46,7 ± 10,2	200	NCy	NNCy
Jetfire	1	47/58	68,1	5	NCy	NNCy
Oregon Cedar x Emerald Sea 1	9	44/59	61,3 ± 12,7	63	NV or NN	NNNV
Oregon Cedar x Emerald Sea 2	7	44/60	61,9 ± 12,4	200	NV or NN	NNNV
	4	57/70	119,1 ± 8,3		NNNV	NNNV
Symptom x Matador 1	2	62/64	127,9 ± 11,9	36	NNNT	NNNT
Symptom x Matador 2	3	39/52	44,3 ± 5,6	53	NT or NN	NNNT
	2	64/76	163,2 ± 15,9		NNNT	
Hillstar x Amadeus 1	1	50/64	83,6	7	NNJ	NNNJ
Hillstar x Amadeus 2	2	44/60	48,8 ± 2,4	30	NJ	NNNJ
Hillstar x Amadeus 3	2	51/69	96,0 ± 7,3	60	NNJ or NNN	NNNJ
TS 174 x N. dubius	5	40/53	45,6 ± 7,0	8	NA	NNAAP
N. x alleniae 1	11	39/48	38,0 ± 4,2	81	VES	VVES
N. x alleniae 2	9	38/41	31,8 ± 6,6	25	VE or VS	VVES
N. x alleniae 3	5	33/47	28,2 ± 4,9	17	VE or VS	VVES
N. x alleniae 4	11	51/54	73,5 ± 11,8	131	VVE or 2VES	VVES
N. x alleniae 5	3	36/42	28,4 ± 0,0	25	VE or VS	VVES
N. x alleniae 6	7	37/44	33,1 ± 10,8	18	VE or VS	VVES
N. cantabricus x Emerald Sea	3	60/68	130 ± 11,2	15	NVC	NVC
El Camino x N. viridiflorus	8	43/50	49,2 ± 6,5	60	N(Cy)VTr	N(Cy)VVTr
Regeneration x Emerald Sea 1	2	70/82	213,5 ± 56,6	29	2NNJV	NNJV
Regeneration x Emerald Sea 2	3	42/55	51,00 ± 8,2	10	NJV	NNJV
Regeneration x Emerald Sea 3	1	31/50	24,5	120	N	NNJV

	9	42/51	44 ± 4,4		NJV	
	3	50/57	74,9 ± 2,2		NNJV	
Regeneration x Emerald Sea 4	5	35/43	28,1 ± 4,7	78	N	NNJV
	1	42/53	48,1		NJV	
	5	52/62	87,8 ± 15,9		NNJV	
Avalanche	3	43/51	50,2 ± 4,2	20	TP	TTP
	9	56/65	108,6 ± 25,5		2TTP	
Sidley x N. assoanus	1	56/67	110,0	4	NNA	NNA
UFO x N. tazetta	1	33/58	33,0	17	N	NNT
	3	46/54	61,2 ± 11,0		NT	
Mowser	1	53/61	89,7	10	NJJ	NJJ
Stratosphere	1	53/67	98,5	16	NJJ	NJJ
Crill	1	36/39	26,5	80	N	NJJ
	4	42/56	52,9 ± 7,9		NJ	
	3	47/67	77,4 ± 6,0		NNJ	
	2	69/77	188,6 ± 34		2NNJ	
Decoy x N. cordubensis	10	42/55	51,5 ± 9,7	50	NJ	NNJ
	1	50/67	87,7		NNJ	
Pinza x N. cordubensis	3	33/48	27,4 ± 2,0	83	N	NNJ
	5	42/54	49,5 ± 9,3		NJ	
TS 35 x N. fernandesii 1	2	32/46	24,4 ± 1,1	41	N	NNF
	4	42/56	50,2 ± 6,9		NF	
	2	53/64	94,9 ± 21,4		NNF	
Altruist x N. fernandesii 4	2	32/50	26,8 ± 2,4	34	N	NNF
	3	41/49	43,1 ± 3,2		NF	
	2	47/60	69,1 ± 6,0		NNF	
Cool Pink	4	60/66	123,7 ± 6,5	26	NNNJ	NNNJ
Harpsichord	4	43/56	55,2 ± 3,3	55	NJ	NNNJ
	6	49/61	76,5 ± 6,2		NN	
Clavichord	2	47/60	68,8 ± 2,4	86	NN	NNNJ
	2	53/63	91,9 ± 3,1		NNJ	
	12	60/67	124,1 ± 17,2		NNNJ	
	4	72/77	211,3 ± 19,3		2NNNJ	
TS 76 x Matador	1	47/61	70,6	15	NN or NT	NNNT
N. cantabricus x Emerald Sea 1	7	53/60	89,2 ± 16,9	19	NVC	NVC
N. cantabricus x Emerald Sea 2	3	46/57	63,1 ± 10	7	**NVC**	NVC
N. cantabricus x Emerald Sea 3	13	47/61	74,1 ± 17,8	62	**NVC**	NVC
N. cantabricus x Emerald Sea 4	3	49/67	84,7 ± 19,9	49	**NVC**	NVC
N. cantabricus x Emerald Sea 5	1	42/58	53,6	1	**NVC**	NVC
N. cyclamineus x Emerald Sea	3	39/45	39,0 ± 1,4	6	**N(Cy)V**	NCyV
	2	47/50	67,3 ± 18,2		**NCyV**	

Z = number of measured pollen grains, a = mean minimum pollen grain diameter, b = mean maximum pollen grain diameter, SD = standard deviation, CPO = chromosome sets of pollen, CPL = chromosome sets of plant, N = number of sprouting pollen per sample, P = chromosome set of N. papyraceus, S = chromosome set of N. serotinus

tetraploid garden daffodils (NNNN) with diploid species (XX). The number of fertile plants is about 1 to 10% of the seedlings for one cross. It seems to be mainly a function of the involved species. High values one finds for tazetta crosses, low values for bulbocodium crosses. For the fertility of pollen you find about 80 to 90 % for species. A value of 6 or 60 sprouting pollen for a sample of the varieties in Table 6 equals a fertility of about 1 to 10 %.

For crosses of garden daffodils with members of the jonquilla section you find 6 with two different pollen types, eight with NX-pollen and two with NNX- pollen. The part of NX- pollen is high for all crosses between garden daffodils and species. The rule seems to be: Cross a tetraploid garden daffodil with a diploid species and some plants produce fertile pollen. Many of them are NX pollen. The same rule one can probably use for XXN which can be generated by backcrosses of NNXX with XX.

Most specialists for genetics are of the opinion all sprouting pollen of NNX plants should be unreduced pollen NNX. This is not the case and it should be the same for other flowers and agricultural crop plants. Besides pollen of NX and NNX you find sometimes N, X and 2NNX pollen.

For the tazetta crosses the volume of NT- pollen is very different for the seed parents Actaea and Loch Coire. This should be the influence of the seed parents. Actaea has a pollen Volume of 57,6 µmm³ , Loch Coire of 101,6 µmm³. Within the tazetta crosses there are to be seen two pollen types with the extraordinary chromosome content 2 NT and 2TTP. The explanation may be: During the reduction division of meiosis arise from the pollen mother cell two cells with half the chromosome content of the plant cell. These two cells both undergo a normal cell division as the cells of a growing plant. The chromosome content remains constant. From each of these all in all four cells four pollen grains develop. When during this process the first step fails, unreduced pollen is produced. When moreover during the second step the separating cell wall doesn't grow, the pollen contains two times the chromosome content of an unreduced pollen.

If the existing fertile jonquilla-,viridiflorus- triandrus and tazetta-hybrids NNXX are combined with tetraploid garden daffodils NNNX- daffodils arise. Many NNNJ have been generated by Grand E. Mitsch and Richard and Elise Havens and NNNV by Bob Spotts. The descendants should be actually infertile.

Picture 3 Symptom x Matador 1

But a great part of the few crosses I made shows pollen fertility. N-, NN-, NX- NNX- and NNNX- pollen are formed, of which the three last are of special interest for hybridizing. Meanwhile it is known, that garden daffodils combined with N. dubius sometimes show some fertility .Steven J. Vinisky proofed it by his Gee Willikers which comes from Hillstar x Pango. Harold Koopowitz found seeds on some of his plants. The daffodil TS 174 x dubius (Table 6) generates pollen with one chromosome set of N. assoanus and one of the standard daffodil TS 174. The problem for me is that the seed fertility seems to show only in warmer climates and the pollen fertility for one plant is not constant for each year. Selfing a seed and pollen fertile plant could give NNAA children, the first fertile allotetraploid daffodils of this form.

In the extreme south of Spain and in Moroco lives the nice autumn flowering natural hybrid N. x alleniae which should be a mule, but about 20 % of the plants are pollen fertile and some set seed. It arose by natural pollination of N. miniatus with N. viridiflorus which grow there together. It can be forecast that in the long run new fertile species will emerge from crosses of different N. x alleniae clones or selfing.

If allotetraploids for example Hillstar (NNJJ) are crossed with diploids for example N. hedraeanthus (HH) daffodils with three different chromosome sets (NJH) come into existence. The only possibility for some fertility of the plant is to generate unreduced pollen. Thousands of NJH seedlings neglected to do this, but this year I found the first fertile seedling with three different chromosome sets. It arose from N. cantabricus x Emerald Sea (Table 6). In the same way seems to behave El Camino x N. viridiflorus, but I am not really sure of the chromosome content of the plant and the pollen.

Up to recently I thought the chromosomes of N. jonquilla and N. viridiflorus would be so similar, that they form bivalents during meiosis. The consequence would be that if you combine one chromosome set of N. jonquilla and one chromosome set of N. viridiflorus to a JV-plant (which is a little complicated) or produce a NNJV-plant from jonquilla hybrids x viridiflorus hybrids they should be fully fertile as crosses of N. poeticus with some N. pseudonarcissus are. My results in Table 6 show, that this assumption is wrong or must be modified. Perhaps only some different chromosomes form bivalents. I often found no fertility at all for Regeneration x Emerald Sea and in other cases many different pollen instead of one NJ(V) – pollen form. This result is disappointing. On this way could have been generated by intercrossing a family of fertile NNJ(V)J(V) hybrids with red and green crowns interesting form and early flowering time.

The breeding of garden daffodils (NNNN) was so successful, because N. poeticus and three species of N. pseudonarcissus could be crossed and fertile diploid and afterwards tetraploid varieties developed. This possibility seems to be not at hand for the rest of the 23 species. Here fertile daffodils must be allotetraploid ,NNXX for crosses with garden daffodils or YYXX for interspecies crosses. First steps into this direction are NNJJ, NNTT and NNTrTr which came accidentally into existence: A unreduced pollen grain of the species found an egg cell of the garden daffodil for NNJJ and NNTT. NNTrTr supposable occurred by the combination of an NTr egg cell with an NTr pollen grain. Targeted the allotetraploid viridiflorus hybrids came into existance. That means we are just at the beginning of daffodil hybridizing. Some possibilities to use the results of the pollen analysis for breeding shall be nominated:

If the species forms some unreduced pollen a combination with garden daffodils leads to NNXX. That means N. assoanus 2 (Table 2) can be used to breed NNAA. This would be the first of this daffodil family. I think a wonderful family with more than one little flower per stem with different colours. For all diploid species should be examined, whether they make some unreduced pollen. Presumably each clone of one species will show another behaviour. A further possibility to generate NNAA daffodils is to combine pollen fertile plants of garden daffodils x N. dubius with seed fertile plants of the same type. Here also daffodils can be expected which possess more or less chromosomes from N. papyraceus.

The unreduced pollen from NNX plants can be combined by backcrossing with the pollen of the species to get NNXX. This should function for Honeybird x N.

hedraeanthus (Table 6), to get the members of the new family of NNHH. The first seedlings sprouted this year. The same can be done for Ufo x N. cantabricus (Table 6) to yield the new class 'NNCC'. Theoretically it should be possible to cross garden daffodils with the tetraploid N. cantabricus foliosus, N. romieuxii or N. bulbocodium and get allotetraploid seedlings. But my effort was in vain till now. The crosses produced no seeds.

NJ-, NT-,NTp- and NTr- pollen can be used to widen the existing spectrum of NNJJ daffodils like Hillstar or Limequilla, of NNTT like Matador and of NNTrTr like Lapwing. Flowers with impressive colours especially red and pink could be yielded. Because Silver Bells supposable has NTr egg cells a combination with NTr pollen is promising. An interesting possibility is to introduce the gene for split coronas into the NNJJ group and get fertile plants. This could be done with NJ pollen of Trigonometry x N. cordubensis (Table 6).

The plants with unreduced pollen NNNJ and NNNT can be employed for backcrossing with NNJJ and NNTT. The results are fertile descendants with the chromosome content NNNNJJ or NNNNTT. Perhaps some daffodils NNNJ with split corona genes of the Mitsch and Havens stable show few unreduced pollen. They could be used with similar results, but a big part of the seedlings should have split coronas. Perhaps the maximum of vitality which is also a function of the number of chromosome sets is not yet reached. It is surely overcome for crosses with 2NNX and 2TTP.

The specified crosses shall originate fertile daffodils which can be of great importance for hybridizers of the future. If the goal is to yield nice daffodils without ideal fertility the different pollen of Table 6 give many possibilities:

- The plants with diploid pollen NJ, NF, NV, NTr, NTp, NT, NP, NC, NCy, NA, VE,and VS can be backcrossed with the diploid or for NV tetraploid species. For the combination with diploid species you get triploid plants which character is formed mainly by the species. If you do this for example with Trigonometry x N. cordubensis a big part of the children will have a split corona. Another possibility is the cross with different species. The plants should have good vitality because of the three participating different species. Furthermore one can combine the diploid pollen with allotetraploid plants which possess two chromosome sets of N and two of different species. The result can be for example a daffodil 'NNJTr' , 'NNJT' , or 'NNVC' with four chromosome sets and therefore good substance. Mixing the diploid pollen with the egg cells of NNNN plants gives NNNX. The influence of the species is reduced but some interesting traits like green colour from N. viridiflorus can show. With N. alleniae for the pollen' VE' and garden daffodils ' NNNN' with much red the children may have a red colour and interesting form of the flower.
- The plants with the triploid pollen NNJ, NNF, NNCu, NNT, NNTp, NNC, NNH, VES, NVC, N(Cy)VTr, NJV can be crossed with other diploid species. The result is for example NVCH or NNJTr which was already achieved from diploid pollen and egg cells of allotetraploid plants, from which there are only few varieties. Here with triploid pollen the possibilities are more numerous. A combination with NNNN makes in most cases no sense, because the influence of the species concerning form and colour is strongly reduced. The

situation is changed if only one trait of the species for example the resistance against a fungus is wished. An exception are crosses with N. x alleniae which come to a daffodil 'NN VES', which with red from NNNN and N. elegans could give nice flowers.

- For the tetraploid pollen NNNV and NNNT backcrossing with the species is perhaps a good possibility. NNJV can be combined with NNNN plant as seed parent. The resulting NNNNJV daffodil is hexaploid and may have red or pink colour , good form and excellent substance.

Not all these combinations should function in practice, but most of them should do. Till now I have made some crosses with Bellsong, Altruist x N. cordubensis 2, 2YR x N. cordubensis, Trigonometry x N. cordubensis, TS 125 x N. assoanus ,Ufo x N. dubius (not listed in Table 6), N. alleniae 1 and Honeybird x N. hedraeanthus. The seed set was good. When the seedlings flower it can be seen if the pollen worked or an open pollination took place. At this point the nuclear DNA content of the plant can be measured and the chromosome content of the used pollen can be calculated. The values for the chromosome content from the volume and the values from the nuclear DNA content should be the same. I am optimistic, that this is the case and that the method shall be successful for hybridizing as well daffodils as other flowers and crop plants.

Literature: (1) T. Sanders, Looking at Pollen, The Daffodil Journal, Vol. 46 Issue 2, Dec. 2009, pages 110-115. (2) B.J.M. Zonneveld, The systematic value of nuclear DNA content for all species of Narcissus L. (Amarillydaceae), Pl Syst Evol (2008) 275, pages 109-132.

Post scriptum (January 2013):
I have some new results about pollen volumes and chromosome contents of daffodils which are written in 'red' into the different tables.

It becomes quite clear that a big part of all NNNX crosses shows pollen fertility. Often one plant has different pollen varieties which can lead to interesting crosses. The NNNJ split corona types 'Harpsichord' and 'Chlavichord' from Richard and Elise Havens shall have many plants with split coronas within their progeny.

The older jonquilla 'Stratosphere' from Grant E. Mitsch and the new jonquillas 'Mowser' and 'Crill' from Ron Scamp are also pollen fertile.

From six seedlings of N. cantabricus of the Sierra de Calatrava x Emerald Sea which flowered in autumn 2012 in the greenhouse five have sprouting unreduced pollen which is extraordinary to my experience for triploid plants with three different chromosome sets.