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Pollen Volume and Chromosome Content of Daffodils; Possibilities for hybridizing 3

If a diploid plant is transformed to a tetraploid in many cases the pollen volume is doubled. The two chromosome sets within the pollen of the tetraploid plant need more place than the one set of the diploid plant .For the variety 'Actaea' which is a tetraploid form of the diploid Narcissus poeticus the values for the pollen volume are about 58 µmm³ and 30 µmm³ (1).

Table 1 Pollen of species

Species	Z	a/b in µm	V ± SD in µmm³	CPO	CPL	Site
N. bulbocodium 1	22	37/52	38,2 ± 10,9	BB	B	Alcofra/Portugal
N. bulbocodium 2	8	38/51	39,8 ± 9,0	BB	B	Evora/Portugal
N. bulbocodium 3	23	34/53	33,9 ± 9,1	BB	B	Bobadela/Portugal
N. bulbocodium 4	11	40/60	49,3 ± 7,8	BB	B	Serra da Estrela
N. bulbocodium 5	44	41/65	59,5 ± 12,9	BBBB	BB	Faro el Picacho/Spain
N.bulbocodium 6	19	49/80	103,0 ± 10,1	BBBBB	BBB	Quiaios/Portugal
N. bulbocodium obesus	11	44/72	72,9 ± 6,6	BBBB	BB	Arrábida/Portugal
N. panizzianus	10	34/45	26,9 ± 2,2	P	PP	Grazalema/Spain

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

The bulbocodiums in Table 1 don't obey this rule accurately. N. bulbocodium obesus is known as tetraploid, for the others the chromosome content is estimated from the pollen volume. I think the rule works best when the polyploids just developed from the diploids. After a very long time the pollen volume of the new plants can reduce because it adapts to the environment. There are better methods to determine the number of chromosome sets within the plant than measuring the pollen volume, for example direct observation and identifying the nuclear DNA content.

The diploid N. bulbocodium 1 from (1) and N. cantabricus from (1) can easily be crossed with jonquilla hybrids like Hillstar and viridiflorus hybrids like Emerald Sea. I think this should be possible too for the diploid bulbocodiums of

Table 1. The tetraploid bulbocodiums produced no seeds or few seeds only crossed with these hybrids till now.

For daffodil varieties and other flowering plants the chromosome sets are known to be one half of the seed parent and one half of the pollen parent. They are fertile when the number of chromosome sets can be divided by two and this gives a whole number. This number denominates the chromosome sets of their pollen or egg cells. In the other case the plants are infertile. Sometimes one finds a little part of sprouting pollen in the flowers of these daffodils. Here the question is which chromosome sets contain the pollen. The answer is important for making crosses. As I wrote in (1) I think that the chromosome sets of the pollen can be calculated from the pollen volume when the parents of the plant are known in the following manner:

Table 2 Pollen of different crosses

Variety	Z	a/b in µm	V ± SD in µmm³	N	CPO	CPL
Gloriosus	20	32/43	22,5 ± 3,6	400	T	TT
Silver Bells	5	41/52	45,4 ± 7,3	23	NTr	NNTr
TS 108 x N. triandrus pallidulus 1	8	42/57	55,9 ± 7,3	12	NTr	NNTr
TS 108 x N. triandrus pallidulus 2	11	36/46	30.4 ± 4,8	25	N	NNTr
	3	42/50	45,3 ± 5,9		NTr	
	2	54/64	95,4 ± 27,6		NNTr	
Altruist x N. fernandesii	13	43/53	52,8 ±13,2	33	NF	NNF
	1	53/78	114,7		NNF	
	1	78/78	248,5		2NNF	
Magic Step	10	42/54	49,9 ± 9,2	20	NJ	NNNJ
Problem Child	17	51/64	84,1 ± 16,8	34	NNJ	NNNJ
Beryl	2	30/45	21,2 ± 5,7	60	Cy	CyPoPo
	20	44/52	52,5 ± 12,9		CyPo	
	6	56/69	111,6 ± 15,5		CyPoPo	
N. romieuxii x Emerald Sea	1	37/48	34,4	29	R	RRNV
	4	61/70	136,7 ± 19,5		RRNV	
	2	68/89	216,6 ± 22,2		2RRNV	
N. x xanthochlorus	11	32/46	24,2 ± 5,0	400	CvV	CvCvVV
N. x alleniae 7	14	38/50	38,4 ± 5,9	83	VES	VVES
	8	52/58	81,5 ± 16,8		VVES or 2VES	

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 the pollen, chromosome sets of the plant, N = number of sprouting pollen per sample

For Altruist x N. fernandesii of Table 2 one mean pollen volume is 52,8 µmm³ . A NNF- plant which in most cases is infertile can sometimes develop a few fertile pollen of the types N, NF or NNF. For N the mean volume is 36 µmm³ and for F 16 µmm³. The sums are 52 for NF and 90 for NNF. The conclusion is that pollen with the measured volume 52,8 µmm³ is NF. It contains one chromosome set of N and one of F.

The validity of this method is sometimes reduced by the statistical spread of the volume values. And furthermore for individual chromosomes the exchange of genetic material by crossing over during the reduction division cannot be excluded.

The parents of Beryl are Chaucer (with poeticus chromosomes) and N. cyclamineus. In the literature you find that it is triploid; it can be CyPoPo or PoPoCy. I decided for the first case because the crown of the flowers shows much red. For crosses this question is of lower importance, because most pollen contain one chromosome set of N. poeticus and one of N. cyclamineus. These can develop as well from CyPoPo as from CyCyPo. Whether the unreduced pollen are CyPoPo or PoPoCy naturally is relevant. In DaffSeek you find that Beryl was used 14 times as seed and 1 times as pollen parent. Much more crosses should be done.

In (1) is shown that NNNX-plants often have some fertile pollen as for example the NNNJ daffodils Cool Pink, Harpsichord, and Clavichord; the last two with split coronas. This trend is to be seen also for Magic Step and Problem Child in Table 2. The pollen types in one plant can be very different. Theoretically N-, NNX-, NNNX-, NN-, and NX- Pollen should be possible. Compared to NNX plants you find much more fertile varieties for NNNX.

This is also the case for tetraploids with three different chromosome sets (XXYZ).They evolve normally from the combination of two allotetraploids with two equal chromosome sets (XXYY x XXZZ) or from an autotetraploid with an allotetraploid which has different chromosome sets (XXXX x YYZZ). An XXYZ plant can develop X-, XYZ-, XXYZ-, XY-, and XZ- pollen. One example is N. x alleniae(VVES), a natural cross of N. viridiflorus and N. miniatus (SSEE) for which I estimated the fertility to be 20 % in (1). Meanwhile, after looking at much more plants in the wild , I think more than 50 % are fertile. N. romieuxii x Emerald Sea (RRNV) belongs also to this group (Table 2). The first two flowering seedlings were fertile. The combination of jonqulla hybrids like Quick Step with Silver Bells (Table 2) yields NNJTr plants. Quick Bells from Frank Galyon is such a daffodil with Silver Bells as the seed parent. I don't know if it is fertile, but the similar cross Ice Chimes x Hillstar from the same hybridyzer shall have some fertile pollen as I know from Steve Vinisky. Here probably NTr pollen from Ice

Chimes were active. Many crosses of jonquilla hybrids with viridiflorus hybrids were made by John Hunter. These NNJV daffodils often are fertile. They form not only fertile pollen but also egg cells. I saw plenty of seed pots on open pollinated plants in the field of John in 2012. My own experiences with the fertility of these crosses were not always so good. An exception from the rule that many NNYZ daffodils are fertile seems to be N. x perezlarae (CvCvES). I looked at many plants of this combination between N. cavanillesii and N. miniatus and no one had fertile pollen.

You find XXYZ plants not only for daffodils but in many other plant families. For Brassica exist three different allotetraploid species from which by intercrossing three different XXYZ types can be created. The most plants show some pollen fertility and develop a few unreduced pollen (2). The authors of this article concentrated on unreduced pollen, but found much more other pollen whose constitution was not examined.

The importance of fertile allotetraploid daffodils was described in (1). Until now exist: NNJJ, NNVV, NNTrTr, NNTT, NNBB? (from Lawrence Trevanion), and VVCvCv. The last is N. x xanthochlorus a cross of N. cavanillesii x N. viridiflorus. These two species often flower together at the same site in Southern Spain. But I never saw this cross there. I think nobody saw it till now in the wild. The description and nomenclature of the flower was made on the basis of a yellowish N. viridiflorus which sometimes can be found. I crossed the two species some years ago and saw the first flowers in autumn 2013. N x xanthochlorus is highly fertile and the pollen grains have a volume as expected (Table 2).

Literature: (1) T. Sanders, Pollen Volume and Chromosome Content of Daffodils; Possibilities for hybridizing 2, January 2013, www.theo-sanders-daffodils.de . (2) A.S. Mason et al. , Production of viable male unreduced gametes in Brassica interspecific hybrids is genotype specific and stimulated by cold temperatures, BMC Plant Biology 2011, 11, 103 (open access).