Theo Sanders October, 2014

Hybrids of different diploid narcissus species can be fertile by generating unreduced pollen

In March 2013, my wife and I met Sally Kington, Brian Duncan, Jan Pennings, and Juan Andres Vargas Braun in Spain near Puertollano looking for narcissus species and found many lovely natural crosses of N. cantabricus x N. triandrus pallidulus. I inspected the pollen of one of these hybrids and saw that 10 % of the pollen grains sprouted in a sugar solution with an addition of Murashige and Scoog basal salt mixture within two hours at 20 degrees C. Because the two parents are diploid and belong to different sections, effective chromosome pairing leading to normal, reduced gametes is very unlikely. Therefore, any good pollen produced by this plants should be unreduced containing two sets of chromosomes, with seven chromosomes from N. triandrus pallidulus and seven chromosomes from N. cantabricus. The pollen fertility of this daffodil inspired me to seek systematically for fertile crosses of different diploid species in 2014 in Spain. To interbreed these plants especially with tetraploid garden daffodils is of great interest.

For 23 Narcissus species – in some classification lists 61 and more species can be found - there exist about 500 possibilities for species crosses, when you distinguish which species is the seed parent and which is the pollen parent. Not all of these possible mixtures will generate offspring and the species to combine in nature must live at the same site and flower during the same time. In DaffSeek so far 73 crosses are listed, some with higher ploidy level than diploid. It is not distinguished which is the seed and which the pollen parent. This can often be not found out in a simple manner.

Most species crosses within a section for example Bulbocodium, Tazetta, or Pseudonarcissus should be fertile, if the ploidy level, the nuclear DNA content, and the number of chromosomes per chromosome set are equal. N. tazetta x N. elegans and many bulbocodium combinations are for example prolific. A few species crosses from different sections such as Poeticus and Pseudonarcissus too give fertile offspring. Nearly all other species hybrids are typically infertile with the modification that for diploid/diploid crosses some unreduced pollen and eggs can develop.

During the trip to Spain in 2014, the following plants were found:

- N. cantabricus x N. triandrus pallidulus = N. x susannae/ N. x litigiosus (near Puertollano), 6 pollen fertile plants of 24
- N. bulbocodium x N. triandrus pallidulus = N. x rozeirae/ N. x fosteri (near Puertollano), 0 pollen fertile plant of 3
- N. hedraeanthus luteolentus x N. triandrus pallidulus = N. x cazorlanus (Santa Elena), 1 pollen fertile and 1 seed fertile plant of 6
- N. hedraeanthus luteolentus x N. cantabricus (near Huertezuelas), 8 seed and pollen fertile plants of 8
- N. fernandesii x N. triandrus pallidulus = N. x incurvicervicus (Sierra Madrona), 1 seed fertile plant of 20
- N. assoanus x N. panizzianus = N. x christopheri/ N. x koshinomurae (near Ronda), 2 pollen fertile plants of 4

Pollen fertility means that the pollen grains sprout in the described solution. For the seed fertile plants a few seed grains could be harvested. Concerning the first cross, for one site it was clear that N. cantabricus is the seed parent, for other sites it could not be decided. I suppose that this has an influence on the possible fertility of the plants. The first three crosses and the fifth cross grew in most cases at a height of about 700 to 800 meters within a distance of 150 km. Most flowers of the parents had damaged anthers with no pollen, perhaps destroyed by frost. Because of this often it could not be decided, whether a hybrid was fertile or not. Perhaps in next spring more information and still better values for the fertility can be measured. N. hedraeanthus x N. cantabricus indicates good seed and pollen fertility. It can be assumed, that the reduction division during meiosis functions nearly as well as for the species parents. For N. bulbocodium x N. triandrus pallidulus it can be supposed that , if more plants should have been found, some too would have been fertile with unreduced pollen. From the other crosses most plants generate unreduced pollen, often about 10 %, or eggs than I expected. It seems to be a good idea to look for the fertility of species hybrids.

I think that this has not been systematically done by hybridizers in the past. Many diploid species have been combined and fine varieties are in commerce. There are for example crosses from N. fernandesii with N. triandrus (Angel's Whisper), N. tazetta with N. poeticus (Laurens Koster, Geranium), N. jonquilla with N. triandrus (April Tears, Fairy Chimes, Hawera), N. jonquilla with N. assoanus (Kidling, Chit Chat) , N. rupicola with N. poeticus (Sun Disc, Sundial), N. tazetta (Soleil d'Or) with N. poeticus (Cyclataz). From these Geranium, April Tears, Hawera, Kidling, Sun Disc, Sundial and Cyclataz are referred to produce unreduced pollen or eggs . I found many viable pollen grains on the anthers of Geranium, but no pollen on Kidling and Sun Disc as described by Helen Link (1). The different climate seems to influence the fertility. Sundial is the seed parent of Kokopelli, Tripartite is April Tears x Baccarat and Freedom Stars, with three named sister seedlings, is April Tears x Canasta, while Pearlie comes from Hawera. Tête à Tête developed from (Soleil d'Or x N. cyclamineus)OP. Soleil d'Or belongs to the tazettas. The open pollination of the seed fertile hybrid was done by N. cyclamineus or another diploid narcissus. The chance to find fertile and beautyful varieties by making crosses is much better than looking for them in nature. Moreover it is not necessary that the two species live in the same district and flower at the same time. The only disadvantage is, that it needs 4 to 7 years for flowering the plants . I combined until now N. triandrus with N. fernandesii. All plants were destroyed by frost during the winter 2011/2012. The mixtures of N. triandrus pallidulus x N. assoanus and N. papyraceus x N. assoanus are growing in the greenhouse, but will need some years to flower. Further interesting combinations should be N. poeticus x N. elegans, N. tazetta x N. cordubensis, N. tazetta x N. fernandesii, N. assoanus x N. tazetta, N. assoanus x N. elegans, N. fernandesii x N. assoanus, and N. assoanus x N. scaberulus. From all these combinations lovely daffodils can be selected and some plants should generate unreduced pollen for further crosses.

In species the normal process during meiosis is the generation of haploid pollen from diploid plants. That means the chromosome number of the parents must be halfed for producing male and female gametes , so that their combination gives rise to the same chromosome number for the descendants as for the parents. For some gametes this process does not function and they are unreduced and diploid as the plant cells. For different species, especially from the crop sector, it was found out that the normal percentage of unreduced pollen and egg cells is 0,1 to 1%. It is variable from one plant to another of a species and can reach for some plants up to 10 or 20 %. The value is influenced especially by the temperature during the development of the gametes which can last some months for narcissus. The unreduced gametes make it possible that tetraploids plants originate from diploids. The first step in most cases is the development of a triploid plant by the combination of the unreduced diploid pollen with the reduced haploid egg cell from another plant. If the occuring triploid daffodil generates some unreduced pollen which finds an haploid egg cell of a nearby plant a new tetraploid plant comes into being. Another way can be that the triploid daffodil forms diploid pollen which combines by selfing with diploid eggs. In one of these ways the tetraploid species for example the tetraploid bulbocodiums BBBB developed from diploid bulbocodiums BB. B is one chromosome set of N. bulbocodium. The process could be imitated by hybridizers: Plants which constitute a high percentage of unreduced pollen must be found. Perhaps a method of seperating the unreduced pollen from the great number of the normal may be developed by process engineering. The best triploid plants can be selected by looking at the number and size of the pollen.

The realisation for the appearance of a naturally occuring allotetraploid plant, for example N. tortifolius, is similar: Two different diploid species, in this case N. assoanus and N. panizzianus, combine to produce a diploid hybrid AP with A being the chromosome set of N. assoanus and P that of N. panizzianus. Some of the new AP plants form unreduced AP Pollen which fuses with a haploid egg cell A of another plant of N. assoanus to make an allotriploid daffodil AAP. If this plant forms unreduced AAP pollen which are brought to the stigma of a PP plant the allotetraploid AAPP is created. An other way can be that an AP – pollen grain combines with an AP – egg by selfing of an AP plant to an AAPP descendant. These procesess should be easier to imitate by hybridizers than the first. If you assume that for flowering of each of the three generations five years are necessary, the development of an allotetraploid plant needs fifteen years. This seems a long time, but on the way to this success many fine daffodils should come into being.

If it is the goal to get XY - pollen you can take it from an allotetraploid plant XXYY, if there exists one, or get it from a cross XX x YY which forms unreduced XY – pollen. With these pollen tetraploid garden daffodils (NNNN) can be pollinated. For example unreduced pollen of N. cantabricus x N. triandrus pallidulus (CT) can be combined with an intermediate 3W-W to produce NNCT with three different chromosome sets. The result should be little plants of a new type with small petals and good substance. Or you intend to develop a small daffodil with a yellow perianth, a red crown, and three flowers per stem with intense fragrance and good substance which shall flower early and have the necessary frost resistance. In this case you pollinate N. assoanus with N. elegans. Both species parents must be first class selected plants. Some of the descendants shall generate unreduced pollen which are combined with an intermediate garden daffodil like Bantam or Cayenne. Most crosses of garden daffodils with species so far are made with diploid species. The outcome are triploid daffodils (NNX) with one chromosome set X of the species. In the case of NNXY (=NNCT above) you get the traits of two different species. The first successful pollinations of tetraploid garden daffodils with XY – pollen were made with N. tortifolius pollen which posesses one chromosome set of N. assoanus and one of N. panizzianus and with N. miniatus pollen which has one chromosome set of N. elegans and one of N. serotinus. In this spring I crossed Rocoza and Lennymore as seed parents with my new N. x xanthochlorus which has two chromosome sets from N. cavanillesii and two from N. viridiflorus and got 10 seed grains. The pollen of N. x christopheri was combined with Laurin and Altruist and gave 34 seed grains. I hope the pollen grains were effictive and not the bees. This is the demonstration, that this kind of pollination is basically possible. You get also seedlings from garden daffodils pollinated with NJ - and NV- pollen from fertile jonquilla- and viridiflorus hybrids as for example Hillstar and Emerald Sea.

Furthermore the combination of XY- pollen with jonquilla- and viridiflorus hybrids should be possible. Here you also obtain tetraploid plants, but in this case with four different chromosome sets. An addional application of the XY-pollen is to put them on the stigma of a diploid species. For example a plant similar to Tête à Tête, concerning the chromosome content, could be developed: At first you cross the Tazetta 'Soleil d'Or' with N. cyclamineus. A seedling which generates a greater number of unreduced pollen is selected and the pollen are used to pollinate N. cyclamineus.

A further possibility to get XY – pollen is to cross tetraploid with diploid species. Tetraploid are N. viridiflorus and some N. cantabricus and N. bulbocodium. I harvested seeds already from cantabricus foliosus x N. triandrus pallidulus, N. cantabricus foliosus x N. assoanus and N. bulbocodium (tetraploid) x N. triandrus pallidulus. Some of the progeny XXY will generate XY – pollen whose production is here favoured over the development of unreduced pollen (2).

For me it is a great pleasure to think about all these possibilities for crosses and imagine the emerging plants. I believe that it is feasable to combine most species with each other to generate diploid, triploid, or tetraploid daffodils with two or up to four different chromosome sets and to forecast their appearance and their character with some accuracy. In reality my possibilities are limited especially by the climate in Germany. Therefore I hope to inspire other hybridyzers or hybridizers to come with better climatic conditions to use these findings for creating exceptional daffodils.

Literature

(1) Link, H. : A Study of Daffodil Pollen. The Daffodil Journal 7 (1970) 67 - 84

(2) Sanders, T.: Pollen Volume and Chromosome Content of Daffodils – Possibilities for hybridyzing 2 (January 2013), www.theo-sanders-daffodils.de