

Looking at Pollen

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[This article originated as a Daffnet posting]

A warning at the beginning. The following text will be a horror for normal daffodil freaks. It is for younger hybridizers only, perhaps up to an age of 80, because the results of the remarks in form of nice daffodils can be seen not until 6 or 10 years in the best case.

If a daffodil has two or four sets of homologous chromosomes it is in most cases fertile. If the two sets of a daffodil are different, as for example in the case of 'Hawera' with one set of *N. triandrus* and one of *N. jonquilla*, you have nearly no chance for fertility. If there are two sets of homologous chromosomes and one different you have a low possibility. This case exists for crosses between tetraploid standard daffodils and most species. In DaffSeek you find in class 7 for Jonquilla Daffodils only few fertile plants of this type: 'Canary,' 'Fruit Cup', 'Mathinna', 'Trevithian', 'Bell Song', 'Tittle Tattle', 'Cherie' and 'Lanarth'. For Triandrus Daffodils, these crosses are listed as fertile: 'Honey Bells', 'Lemon Drops', 'Harmony Bells', 'Silver Bells', 'Niveth', 'Rippling Waters', 'Robert Berkeley' and 'Thalia'. Some of these daffodils have no registered descendants, most of them one or few. I think some more daffodils with this chromosome constitution should be fertile, but nobody looked at the pollen.

If you do this, you have three possibilities:

*The anthers look dry and you see no pollen. This means the flower is not fertile.

*Pollen mass is to be seen with the naked eye; under the microscope you find deformed or very little pollen corns or good looking pollen corns, which do not sprout.

*Pollen mass is to be seen. Under the microscope a good part of the pollen corns looks fine and some of them sprout, if treated in the right way.

The method of causing the pollen to sprout, and examining it under a microscope has been described by Laurence Trevanion in 'A Look at Pollen, *The Daffodil Journal*, Vol. 37, No.2, December 2000, pages 90-102'. He proposed a sugar solution as a medium for sprouting the pollen. In most cases this works well, but some pollen show no signs of life. I found out that these pollen may grow, if you enrich the sugar solution with an addition of the Murashige and Skoog Basal Salt Mixture, which is used for tissue culture and which you can get for example from 'Sigma-Aldrich Fine Chemicals'. I dissolve 1.4 g of sugar and 20 mg of Murashige and Skoog medium in 20 ml of deionized water. These exact amounts are not really necessary. You can take an eggcup, fill it with deionized water and give to this a quarter of a teaspoon of sugar and a knifepoint of Murashige and Scoog Medium. A drop of the solution is given on a glass slide, and pollen is moved on the point of a match from the anthers to the drop. The slide is kept at room temperature in a closed plastic box with water on the ground (see picture), to reduce evaporation from the drop. Two hours later you can see the sprouts with a microscope, if the pollen is fertile. The microscope you can buy at a low price in a shop for toys.

In this year I found four fertile daffodils from the following crosses in my seed beds. The number of sprouts within a drop is given in brackets: 'Altruist x *N. cordubensis*(8), 'Loch Leven' x *N. cordubensis*(2), unknown standard daffodil x *N. cordubensis*(10), (('Pontresina' x ('Edna Earl' x 'Rashee')) x *N. assoanus*(30). The first question is: Which composition has the pollen? The second,: Which crosses are interesting?. It is not plausible, that the plants should be tetraploid of the type NNJJ and the pollen of NJ. N is the chromosome set of the standard daffodil and J that of the Jonquilla. This case is seldom and the number of sprouting pollen corns would be greater. It is more probable that the plants are of the type NNJ, which is the normal case. Very seldom fails the reduction division during meiosis with pollen NNJ as the consequence. Furthermore the pollen can be N, which is not interesting for crosses or NJ, with little variations concerning the number of J- chromosomes per set. For the

best case scenario 'NJ' exist many interesting possibilities for crossing.

- If the listed daffodils are seed fertile too with NJ egg cells, they can be crossed with each other. The products would be tetraploid daffodils of the form NNJJ like 'Hillstar', 'Limequilla', 'Quick Step' and some others but with a greater spectrum for colours. The disadvantage is: If the seed fertility is as low as the fertility of the pollen, many flowers would be needed to get some seed corns.
- Successful should be the generation of fertile tetraploid daffodils by crosses with the existing sorts like 'Hillsta', 'Quickstep', 'Regeneration', 'Pink Step'. Perhaps the children have red or pink cups. Similar crosses can be made for example with 'Emerald Sea' (NNVV) and its relatives with two chromosome sets of standard daffodils and two sets of *N. viridiflorus*. The children are NNJV which should be fertile like NNJJ, because both V and J belong to the Jonquilla Group.
- All diploid species can be combined with NJ- pollen for example *N. triandrus* (TrTr), which gives NJTr like the new 'Crystal Bells', 'Euphonic Bells' and 'Meticulous' from Mitsch Daffodils, which however come from 'Limequilla' x *N. triandrus*. With three different chromosome sets they should have more vitality than JTr a cross of *N. Jonquilla* with *N. triandrus*. All these crosses are infertile.
- 'Matado' (NNTT) with two chromosome sets of standard daffodils and two sets of *N. tazetta* gives with NJ-pollen NNJT descendants.

The same speculations made for fertile NNJ can be done for NNTr, for example 'Silver Bells', which is fertile and I think in many cases forms NTr-Pollen and egg cells. Equivalent crosses as with NNJ can be made. The result of 'Silver Bells' (NNTr plus one chromosome) crossed with NJ-pollen from NNJ should be NNTrJ. 'Spring Chimes' and 'Quick Bells' may have the same constitution but their parents are 'Silver Bells' and 'Quickstep' (NNJJ). Cross a diploid species with a tetraploid standard daffodil or a tetraploid species like *N. viridiflorus* and you get some

fertile plants, which you can use for the described crosses and many others.

I know that Peter Brandham writes in his article 'Triploidy in Narcissus, the Fertility Debate' (*Daffodils 2002-2003*): '...the probability that allotriploids will produce viable haploid or diploid gametes falls practically to zero'. But 'Silver Bells' (NNTr plus one chromosome) crossed with *N. triandrus concolor* (TrTr) has 'Ice Chimes' as a descendant with 22 Chromosomes, which can only be NTrTr plus one chromosome. Two further descendants from 'Silver Bells' OP are 'Lapwing' and 'Mission Bells'. With 29 chromosomes they are NNTrTr plus 1, which surely comes from self pollination of an NTr egg cell with an NTr pollen. For the effect of NJ pollen there are no examples in DaffSeek. But in any daffodil to be generated it should be possible to proof by measuring the nuclear DNA content, that NJ pollen and other NX pollen are produced and effective for some NNJ or other NNX (allotriploids) in a higher rate.

In autumn 2007 and 2008 I saw many *N. x alleniae* in Southern Spain. The pollen of some plants looked good, observed with the naked eye. The chromosome constitution is VVM, in which M stays for one chromosome set of *N. miniatus*. *N. miniatus* is originally composed of two chromosome sets of *N. serotinus* and two sets of *N. elegans*, an autumn flowering Tazetta. The chromosome set of *N. elegans* consists of 20 chromosomes, which divide into two groups of 10 during meiosis. To be seen are four groups of 5 chromosomes. This special situation may support some fertility. I found two plants each with ten sprouting pollen under the microscope. If the pollen is VM there are many interesting possibilities for crosses. Standard daffodils with red or pink crowns may be a partner. The descendants can be plants with red in the corona, some flowers per stem, flowering perhaps in autumn. Crosses with NNJJ and 'Silver Bells' are further possibilities. They should have more flowers per stem than the foregoing group and be of good vitality and attractive form.

The combination of standard daffodils (NNNN) with *N. dubius* (JJJJPP) leads to NNJJP. P means a chromosome set of *N. papyraceus*. Because the plants possess only one P; they are in most cases not fertile. .But the situation is not too bad, because the two sets

of N and J have no problems to divide by reduction division during the process of meiosis. I found this year one seedling of 'Ufo' x *N. dubius*, which showed ten sprouting pollen under the microscope. In 2007 I saw one sprouting pollen from ('Patriarch' x 'Rockall') x *N. dubius*. In most cases the pollen should be NJ or NJP. Crosses with tetraploid sorts like 'Pink Step' with 'Matador'. or Poeticus Daffodils and with *N. triandrus* can have interesting results.

I think most of the descendants of the proposed crosses should be hardy enough for our winters. Some, especially the autumn flowering types may grow better in a more Mediterranean climate.

I would be delighted, if other hybridizers would find more fertile daffodils under their new seedlings or listed daffodils, which were thought to be infertile till now. Further progress in daffodil breeding should be the consequence.