# The Fuchsia Breeders Initiative

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Contributions for the next issue, which is scheduled for the end of December 2015, should be in the editor's possession ultimately on 1 December 2015.

Please send your contribution in Word, with the photos attached separately. Large contributions can be transferred by uploading the file by e.g. WeTransfer

> Photo on front page: F. triphylla PB7760#7'



# High temperatures have both advantages and disadvantages

Again, weather conditions have had their ups and downs this year during the first part of the fuchsia growing season.

On average, May and June have been rather cold months, inducing growth and flowering of the fuchsias lagging behind for several weeks. At the end of June, cold weather conditions suddenly changed, and turned into a heat wave with duration of 10 days. The maximum temperature reached was even 37 degrC, while the maximum temperature ever measured in The Netherlands amounts to 38.6 degrC. Most fuchsias in my garden have survived, but many have suffered (especially the direct Hemslevella offspring) or have even been severely damaged. Flowers have become smaller, many colours have become very different -at least temporarily- and several fuchsias had their flowers 'cooked' in their buds, even at shady positions.

However, for Fuchsia hybridists such weather conditions also have their advantages. It allows us to learn about which cultivars are able to withstand high temperatures for a prolonged period of time, even when placed in full sun. The discoloration of flowers could tell us something about anthocyanin pigment levels. And furthermore, at such high temperatures substantially more unreduced gametes are produced than at lower temperatures, which could give rise to very different results in our crossings. Therefore, one of my first activities in the weeks following termination of the heat wave was to perform as many crossings as possible, trying to rip the potential advantages of the high temperatures.



Editor of The Fuchsia Breeders Initiative

Mario de Cooker

A lot of copy was available for this issue of The Fuchsia Breeders Iniative. Therefore, choices had to be made. The article on producing Triphyllas with bronze foliage has been postponed to the December issue. In its place, a couple of articles on the use of Flow Cytometry in Fuchsia hybridization have been included. This technique provides us with a better understanding of the results of our Fuchsia crossings, at least if these are not too far away from the species. Also Mr. Edwin Goulding's presence has continued with an article on his ideas about Encliandras hybridization.

The networking in relation to the articles in TFBI is starting to develop and grow. I had several interesting e-mail discussions on various subjects related to fuchsia hybridization. By this, new ideas and approaches may surface and strike root. So, if you have any questions, remarks, ideas, criticism, or whatever else might be of interest: don't hesitate to put it forward, or have it published in TFBI!

Maris de Corher.

# In search of the white F. triphylla

By Mario de Cooker

Photographs: Mario de Cooker

# Part 4: The genotype of the F. triphylla flower color

In 2010 Mr. Hans van der Post, a Dutch fuchsia hybridizer, succeeded in creating a soft pink *F. triphylla* as a selfing from *F. triphylla* "Herrenhausen'. A couple of years earlier, new *F. triphylla* seedlings, the *F. triphyllas* "PB 7760 # xx", raised from seed provided by Dr. Paul Berry, became available for hybridization purposes. Together they provide the basis for creating a new series of (near) white *F. triphyllas*.

#### 1. Introduction

In the previous issues of The Fuchsia Breeders Initiative the experimental setup and first results of the programme for creating a white *F. triphylla* have been described. In this issue, on basis of the results of the *F. triphylla* crossings made, a plausible representation is derived for the *F. triphylla* flower color genotype.

The major part of data interpretation in this paper, specifically the calculations and analysis, has been performed by Mr. Henk Waldenmaier. Grateful thanks are due to all his help and assistance, his constructive criticism and the work performed.



Fig.1: Three types of flowers have been obtained in the F. triphylla F2 generation: pale pink, mixed orange/pink and entirely orange.

# 2. Experimental results of the first two growing seasons of the *F. triphylla* F2 generation

Extensive information on the experimental setup and results of the *F. triphylla* crossing programme can be found in the previous issues of TFBI.

In the end, 110 seedlings of the *F. triphylla* F2 generation have flowered after 2 growing seasons, which is some 40% of the original seedlings. Several causes have contributed to this relatively small and rather disappointing figure. A large part of the seedlings has died because of a diversity of reasons, e.g. by botrytis, by attack of vine weevil, or simply because they were not able to survive low temperatures during the winter season in the glass house. Furthermore, part of the seedlings may have been generated from unbalanced

(aneuploid) gametes, resulting in structural weakness or inability to produce flowers (see the text box below for explanation). Indeed, frequently weak growing has been observed in the *F. triphylla* progeny, as well as virus like symptoms in the leaves.

Three types of flowers have been obtained in the F2 generation.

I. F2 seedlings which have flowered, and having light green colored foliage, have <u>all</u> produced white to pale pink flowers in various shades of pink, with all kinds of flower shapes.

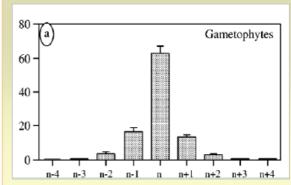
The F2 seedlings with darker foliage have produced two types of flowers:

II.1 Tube, sepals and corolla all orange; various shapes of the flower;

II.2 Outside of the tube and sepals orange, inside tube pink; pink corolla in

#### Aneuploidy

Aneuploidy is the deviation from one to several chromosomes in the cell nucleus from the normal quantity of 2n. It is a phenomenon that both autopolyploid and allopolyploid plants frequently possess. See the following figure from a review study [1] for the frequency of occurrence of the ploidy of pollen grains. The average frequency of euploid gametes in autopolyploids is only 64%, for alloploids it is even less, only 57%. The occurrence of aneuploid seedlings is, therefore, the rule rather than the exception. Often these are also reasonably fertile.



From this frequency distribution it follows that the probability of a polyploid crossing (assuming that the oocytes could also have a similar distribution) delivering 2n progeny could be less than 50%. This is something to take into consideration in our own *Fuchsia* breeding work.

various shades of pink or mixed pink/orange/red; various shapes of the flower.

See Photo 1 on p. 2 for the three types of *F. triphylla* flowers.



Photo 2: Berries produced by orange F. triphylla



Photo 3: Berries produced by pink F. triphylla

# 3. Analysis of the genotype of the *F. triphylla* flower color

3.1. Data obtained from the crossing programme

In total, 110 F2 seedlings have flowered. All of these seedlings have been taken into account in the genotype calculations.

15.5 % of the F2 seedlings has a pale pink tube and pink sepals (the type I flowers, all in various shades of pink). All of these seedlings have a light green underside of the leaves, light green twigs as well as light colored berries.

16.4 % of the F2 seedlings (the type II.2 flowers) has an orange tube, orange

sepals and various shades of pink and pink/orange/red colors in the corolla. All of these seedlings have also a dark underside of the leaves. The flower buds of such seedlings start growing with a pale pink color, which gradually changes into orange. Sometimes a pale pink shade is retained in parts of the tube.

The remainder of the seedlings all have orange (type II.1) flowers.

See Photo.2 and Photo.3 for the various types of berries of differently colored *F. triphylla* seedlings.

Both length and shape of the tube show large variations, however not depending on the flower color. Only the flower color has been taken into account in the genotype calculations.

#### 3.2. Assumptions on genotypes

In the wild, *F. triphylla* exhibits considerable morphological variability, including the flower color [2], which ranges from pure orange to orange-red grada-



Photo 4: F. triphylla "PB7760-#7"



Photo.5: F. triphylla 'HvdP'

$$R_6$$
 $R_4$ 
 $R_5$ 

Fig.1: Backbone of anthocyanins

tions. For the relevant part of the *F. triphylla* color genotype, e.g. for seedling PB7760#7, it is therefore assumed that it can be represented by **AaaaBBBB** for the 4 sets of chromosomes of the tetraploid *F. triphylla*.

In this representation of the F. triphylla genotype, the 'a' describes the allele encoding for absenteeism of hydrolysis on R3 of the anthocyanin molecule (see Fig.5), 100% 'a' resulting in a pure orange color. 'A' encodes for hydrolysis on R3, resulting in a pink or red color. Because of the color variation in the species it is assumed that on one of the loci hydrolysis on R3 has occurred. These colors are reflected in the flower phenotype only in presence of the locus for anthocyanin production 'B'. In case of full absenteeism of 'B' (so for a homozygous 'b'), the anthocyanin production is fully interrupted, resulting in a white flower if no other genes would influence the flower color.

All in all, 100% 'a' results in a pure orange flower. The higher the percentage of 'A' (25%, 50%, 75%, 100%), the more the color will shift to the pink side of the spectrum. In case of methylation on R3 (which is very likely to have occurred in the species) the pink will turn into red.

Extensive information on the Fuchsia flower color can be found in [3].

If by mutation, one of the alleles 'B' is lost, the *F. triphylla* genotype AaaaBBBB changes into **AaaaBBBb**. In the genotype of *F. triphylla* 

Table 1.

Crossing: F. triphylla 'HvdP' x F. triphylla 'PB#7' Aaaabbbb x AaaaBBBB	
F1 genotype	Frequency (%)
AAAAbbBB	0.01
AAAabbBB	1.03
AAaabbBB	24.0
AaaabbBB	48.9
aaaabbBB	26.1

'Herrenhausen' such mutation, most probably a deletion mutation, has taken place. As *F. triphylla* 'PB 7760#7' has considerably better growth and winter survival properties than *F. triphylla* 'Herrenhausen' and its descendant *F. triphylla* 'HvdP', this deletion mutation has presumably resulted as well in the deletion of some more genes other then those responsible for anthocyanin production only. From the genotype



Corolla of pink F. triphylla showing various shades of pink.

AaaaBBBb, by self fertilization a pale pink to white flower can be produced in case of the occurrence of 'Double Reduction' (see text box on p.5 for explanation). It is therefore assumed that the *F. triphylla* 'Herrenhausen' flower color is represented by such genotype **AaaaBBBb**. From this species, by self fertilization *F. triphylla* 'HvdP' has been obtained. Its phenotype (see Photo.5) suggests that anthocyanin production is very low. It is therefore assumed that its genotype can be represented by **Aaaabbbb**.

The color and color intensity are deter-

mined by the percentages of the respective 'a' or 'A' and 'b' or 'B'. The genotype Bbbb encodes for a weakly colored flower, while a BBBb flower has strong coloration.

It is not unlikely that still more genes might play a certain role in the flower color. This has not been taken into account in this article as the quantity and quality of the data do not allow such detailed discrimination [4].

# 3.3. Calculations on the genotype of F. triphylla F1 and F2 generations

Calculations have been performed with the computer programme F1GenCalc [3]. For Double Reduction, a figure of 30% has been assumed for both loci A/a and B/b (although, for A/a it could of course be absent).

Coupling of loci, if any, has not been taken into account. Also the occurrence of FDR and/or SDR (see text box on p.5 for explanation) has been ignored, although this will have undoubtedly taken place to a certain degree.

#### 4. Results and discussion

#### 4.1. The F1 generation

F. triphylla 'HvdP' does not produce any pollen. Therefore, making an F1 generation by self fertilization was not possible. One more step was needed to create white/pink progeny.

The F1 generation has been produced by making the crossing *F. triphylla* 'HvdP' x *F. triphylla* 'PB7760#7':

#### Aaaabbbb x AaaaBBBB

The resulting F1 genotype distribution is provided in Table 1 (page 4). As can be seen, the locus for encoding the anthocyanin production is in all cases represented by bbBB.

As all 14 seedlings of the F1 generation have an orange tube, orange sepals and an orange corolla, it can be concluded that for producing a white/pale pink or

combined pink/orange flower, presumably less than two B-alleles for anthocyanin production should be present. Then at least the combination bbbB, or even bbbb seems to be imperative as the B locus composition for a mixed pink/orange or white/pale pink phenotype.

All F1 seedlings have proven to be vigorous F. triphylla specimen.

#### 4.2 The F2 generation

In the F2 generation, many pale pink seedlings show up. In general, these seedlings have far better growth and overwintering properties than their female ancestor *F. triphylla* 'HvdP'. Evidently, by crossing over during meiosis in producing the F2, (part of) the deleted genes encoding for vigorous growth and low temperature survival properties during winter storage have been restored in the progeny, while the defect for anthocyanin production has been preserved [7, 8].

If we would be interested only in locus B (i.e., the locus which represents the encoding for the anthocyanin production) as a measure for describing the white/pale pink and pink/orange flowers, the calculations for assessing the F2 generation would be simply related to the genotype calculation

#### BBbb x BBbb

Assuming, as a first approximation, that the white/pale pink flower would be represented by the genotype bbbb, and the orange tube/pink corolla flowers by Bbbb, then it follows from the calculation for this crossing that the overall number of white/pale pink + orange/pink flowers amounts to 3.3 + 23.1 = 26.4% [5]. This figure is in reasonable agreement with the figure of 31.9% which follows from the measurements. The relative amounts, however, do not match the ~ 1:1 ratio as is found for the seedlings.

Such simple approach therefore needs some further elaboration.

As inheritance in polyploids is mostly of a quantitative nature, it is not unlikely that also the colors of the flower are quantitatively additive. Then the phenotypes of the F2 generation will exhibit a gradual change, starting at the genotype aaaabbbb as the best white colored flower. In such case, a reasonable assumption is, that for the white and pale pink phenotypes also the adjacent most nearby recessive colored genotype: aaaaBbbb has to be taken into account. The calculations should then be extended to include also this genotype in the outcome of the F1 crossings delivering white/pale pink flowers.

In the experimental programme, the F2 generation has been produced by making arbitrary combinations of different F1 seedlings, also including some selfings.

From the calculations it follows that the share of AAAABBbb and AAAaBBbb in the F1 genotypes is very low. These have therefore been ignored in further calculations. Relevant genotype combinations (male and female are assumed to be interchangeable) to be included are:

AAaaBBbb x AAaaBBbb AAaaBBbb x AaaaBBbb AAaaBBbb x aaaaBBbb AaaaBBbb x AaaaBBbb AaaaBBbb x aaaaBBbb

From the calculations it follows that the relative weighted amount of the aaaaBbbb F2 genotype produced by these combinations (as stated: presumably also representing part of the white/pale pink phenotypes) amounts to 7.5%.

As the overall white/pale pink + orange/pink genotypes amount to 26.4% (see page 4), it then follows that:

• the share of white/pale pink flowers = 3.3 + 7.5 = 10.8% (vs. 15.5% meas-

ured);

the share of orange/pink flowers
 = 23.1 - 7.5 = 15.6% (vs. 16.4% measured).

Although these figures are in good (the 15.6 vs 16.4%) to reasonable (the 10.8 vs 15.5%) agreement with the observations, the share of white/pale pink flowers in the calculations seems still to be somewhat underestimated.

An additional observation could, however, also be taken into account. Already in an early stage of growth of the seedlings, the white/pale pink flowering seedlings can be discriminated on basis of the color of the underside of their leaves and their overall appearance. Buds or flowers need not to be present. For the orange/pale pink seedlings, discrimination on such criterion appeared to be impossible, as at least the color of the growing buds had to be known.

From the original seedlings, 11.6 % could be selected already as potential 'whites' in an early stage on basis of the leaf color [6]. This figure is in excellent agreement with the calculated figure of 10.8%.

#### 5. Summary and conclusions

In this paper it has been assumed that the genotypes of the tetraploid *F. triphylla* species, used in the intraspecific crossing experiments, can be characterized as:

F. triphylla 'PB 7760#7".....AaaaBBBB

F. triphylla 'Herrenhausen'....AaaaBBBb

F. triphylla 'HvdP'.....Aaaabbbb

In these genotypes

'a' represents the recessive trait encoding for absenteeism of hydrolysis on R3 of the anthocyanin molecule, resulting in the color orange;

'A' represents the dominant trait, derived

#### **Double Reduction**

In the meiosis: the process of formation of gametes (the pollen and egg cells), the chromosomes duplicate into a pair of joint chromatides, and come together for pairing and exchanging part of their genetic material.

In diploid specimen, the chromosomes pair up in bivalents, which after pairing separate during the first division. Subsequently, the chromosomes split up the chromatides into separate chromosomes in the second division. In this process, identical alleles on the chromatides, originating from the same chromosome, eventually end up in separate gametes.

Pairing of chromosomes of polyploid specimen in the meiosis does not only occur as bivalents, but also trivalents and quadrivalents can be formed. By this process, identical alleles on the chromatides of a specific chromosome may end up in the same gamete. This process is called 'Double Reduction'.

### First and Second Division Restitu-

One of the main reasons for 2n gametes formation is meiotic nuclear restitution. It is defined as the formation of a single nucleus with unreduced chromosome number, and the failure of the first or the second meiotic division

If in the meiosis the first division does not proceed properly, the paired chromosomes are not separated into different cells. This is called 'First Division Restitution'.

If the chromosomes have properly separated in the first division, each of the two daughter cells from the first division divides again to produce a total of four daughter cells, each having just a single set of chromosomes. An improper division might however occur in the second division. This is called Second Division Restitution'.

The outcome of both First and Second Division Restitution is, that the gametes formed don't have the haploid chromosome number, but the double amount. These are the unreduced gametes, by which from a diploid specimen polyploid varieties may be produced.

by hydrolysis on R3 of the anthocyanin molecule, shifting the color to pink, or red if also methylation has occurred;

'b' represents the recessive trait for interruption of anthocyanin production;

'B' represents the dominant trait for anthocyanin production.

The experimental results can be satisfactorily described by assuming a quantitative additive color inheritance pattern. The white/pale pink *F. triphylla* flowers, exhibiting a gradual change from near white to pink, can be characterized by the genotypes aaaabbbb and aaaaBbbb.

Other genes might (and most probably will) also play a role in producing the flower color. The quantity and quality of the data, however, do not allow further elaboration on this.

#### References and remarks

[1] J.Ramsey, D.W.Schemke, 2002; Neoploidy in Flowering Plants; Annu. Rev. Ecol. Syst. **33**: 589 – 639. [2] P. E. Berry,

'The Systematics and Evolution of Fuchsia Sect. Fuchsia (Onagracea)', in Annals of the Missouri Botanical Garden, Vol.69, 1982. (p. 188).

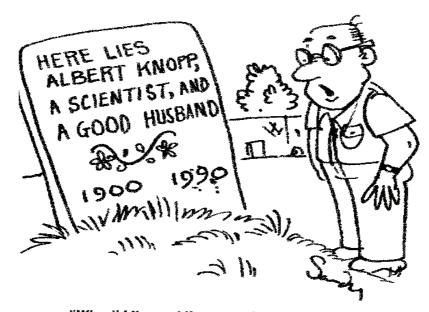
[3] H. Waldenmaier; information contained on website

http://members.home.nl/henkwalden maier/

- [4] As is suggested by the phenotype of numerous *Fuchsia* cultivars, different genes could still play a separate role in producing the color of the tube, the color of the sepals and the color of the corolla. This, however, cannot be derived from the experiments.
- [5] On request, detailed information on the genotype frequencies resulting from the calculations can be obtained from the author.
- [6] In the experiments, a first selection of young seedlings has indeed been performed this way (see TFBI, issue 3, page 13). <u>All</u> selected seedlings having light green leaves which have flowered,

have produced pale pink flowers.

- [7] In the F2 generation, the pale pink and mixed orange/pink seedlings have better vigorous growth and better winter survival properties than *F. triphylla* 'HvdP'. As yet, however, the superior properties of both *F. triphylla* 'PB7760#7' and the F1 seedlings have not been met. By making crossings F2 'Pink' x *F. triphylla* 'PB7760#7' and subsequently making selfings, this could probably be further improved.
- [8] A comparable event may have occurred in producing F. 'Our Ted' by the crossing 'Thalia' x 'Thalia'. In the pentaploid 'Thalia', with most probably the genome TTTTF (T=F. triphylla, F=F. fulgens), the defect for anthocyanin production could have been introduced by an unreduced gamete from F. triphylla (to be published). Also 'Our Ted' is a relatively difficult to grow Fuchsia cultivar, but its progeny (e.g., 'Strike The Viol') are vigorous growers, while still some tendency for producing 'white' has been preserved.



"Why did they put three men into one grave?"

Data processing and interpretation is difficult, not only in Fuchsia genetics.

# Flow cytometry: a useful tool in Fuchsia hybridization

By Mario de Cooker

#### **Abstract**

The article describes in short the Flow Cytometry technique for measuring DNA values in different organisms. It considers the possible use of this technique in Fuchsia hybridization.

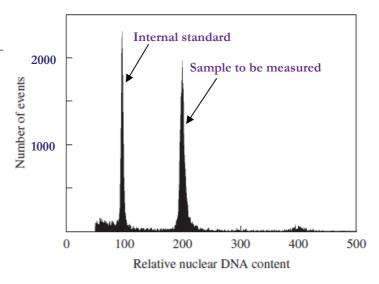
#### What is Flow cytometry?

Flow cytometry is an analytical technique that allows us to find out in a relatively simple and inexpensive way with a flow cytometer (a particle meter), the amount of DNA in cell nuclei. In different organisms (plants, animals, and humans), the DNA in a cell nucleus is often different from others. Allowing for the necessary limitations, the technique can be used in the identification of, for example plant species.

In brief, the technique means that the DNA in the cell nucleus is stained with a fluorescent substance, which quantitatively binds to the DNA. The nuclei are then passed through the flow cytometer, where they are illuminated with light from a laser source. This allows the nuclei to fluoresce. The amount of fluorescence is recorded for each nucleus. As a result of the quantitative bond between the DNA and the dye, the strength of the fluorescence is used to measure the amount of DNA present in the nuclei. The number of cell nuclei is then measured with a computer as a function of the amount of fluorescence. In this manner a picture of the relative amount of DNA in the nuclei of the specimen (eg. a particular species or crossing) is obtained relative to an internal standard in the measurements used.

This analytical technique is still relatively new. The first successful experiments were carried out in 1973 but it took until 1983 before a good enough method was developed for measuring the DNA in the cell nuclei of plants. Thereby, a small quantity of fresh material from the plant (usually a small piece of a leaf) is chopped into pieces and the cell nuclei, in an appropriate buffer, are isolated and stained with a fluorescent substance. Because the equipment has improved so much over the years, results are now more reliable, and analysis costs have decreased significantly.

For additional information refer to the literature sources [ 1 ] and [ 2 ].



# The absolute amount of DNA in a cell nucleus, the 2C-value.

The purpose of the flow cytometry measurements is to establish the absolute amount of DNA in a non-dividing cell nucleus. This means the size of the full undivided genome in picograms (pg) or mega base pairs (Mbp). 1 pg = 986.9 Mbp so giving 986.9 million base pairs. This DNA measurement is referred to as the 2C-value. For a diploid plant the 2C-value corresponds to two sets of chromosomes; for a triploid plant the 2C-value corresponds to 3 sets of chromosomes; for a tetraploid plant to 4 sets etc..

As indicated above, the 2C-value shown in the flow cytometry measurements is not determined directly, but relatively to an internal standard: another plant with a known amount of DNA. This internal standard does not always have to be the same, but is adjusted to the value of the DNA in the cell nucleus of the samples to be investigated. If the absolute amount of DNA of the internal standard is known, from measurements, the absolute amount of DNA from the target plant can be calculated. This 2C-value, and not the relative value, is needed if we are to compare DNA levels from different sources, eg. a series of measurements which use different internal standards.

#### What is the 2C value used for?

In the first place, knowledge about the value of 2C-organisms is of scientific interest.

In 2003, Kew Gardens in England launched a series of workshops with the aim of setting up a database for the

worldwide establishment of the genome of all plant species (see http://data.kew.org.cvalues/ for more information). This sort of information helps us to gain insight into how DNA functions in certain organisms (whereby the absolute size of the genome is certainly not the determining factor).

Furthermore, the intention of the workshops was to agree on definitions, eg., the terminology (what is a clear definition of the C-value, what is 2C, etc.) and the procedures. In particular, it was of great importance to agree on the use of a limited set of internal standards in the flow cytometry measurements of plants. All this improved the degree of accuracy and comparability of measurements. The results of the different workshops, the last held in 2007, show that this was no easy matter. Specialist horticultural terminology on the subject is still hard to find; therefore, be warned when consulting different information in sources of literature.

Flow cytometry is widely used in a practical sense for the (commercial) breeding of plants; in particular for the determination of the amount of DNA and ploidy, and any ane-uploidy in the programme's products.

In the Netherlands the company Iribov BV provides analytical services on a commercial basis to plant breeders (http://iribov.com/nl/analytical-services). The accuracy claimed for the flow cytometry measurements is +/- 1%, which corresponds roughly to the difference of a chromosome.

# The accuracy of measurements and possible sources of error.

In some cases, the accuracy of the measurements could be negatively influenced for several reasons. In literature a number of possible sources of error are reported.

- . Outdated or poor equipment. This may cause drift differences between measurements run on different days or at several different times. This will not play any role anymore in companies offering commercial services.
- Differences in 2C-values can occur in the same species from different sources. The literature on this subject is ambiguous. A number of differences, found initially coming from species, are now attributed to measurement errors [9]. This could have hidden real differences in plants of the same species, eg. as a result of aneuploidy in the gametes of the species. Especially in the polyploid species such differences can easily occur. Close attention should be paid to any measurements where clear differences exceed the expected accuracy of results.
- . The use of the wrong plant-material speaks for itself, and will undoubtedly occur sometimes.
- . Faults can occur in the plant samples. The material may be contaminated, for example with chemical compounds, which

can cause additional staining or otherwise disturb samples and their results. This seems however not to occur very frequently in practice. It is advised that, following flow cytometry measurements, chromosome counts should also be performed as a final check. This is however both expensive and time consuming, and not financially viable for non-commercial breeders such as Fuchsia enthusiasts. Lastly, it is advisable to carry out important measurements more than once.

- . The use of waste plant material. Actually, this plays a role if plant material is taken over long distances or needs to be transported for long periods of time, for example, with field research in remote locations.
- . The use of different, possibly less suitable, internal standards. Because the measurements of the relative amount of DNA are determined relative to an internal standard, there must always be a conversion of the measured values if we want to have the absolute amounts of DNA. Certainly, in the past, we might have encountered differences of up to about 20% between measurements that were carried out in different laboratories. Nowadays it will play less and less a serious role.

Visually, differences can often be observed between a diploid and polyploid (e.g., a tetraploid) *Fuchsia* specimen. Clear evidence should however still be delivered by using adequate techniques such as flow cytometry.

The photograph shows a diploid (on the right) and tetraploid form of *F. fulgens* var. *gesneriana*.

From flow cytometry measurements [ 3 ] it follows that

2C value for diploid *F. fulgens* var. *gesneriana* = 4.0

2C value for tetraploid *F. fulgens* var. *gesneriana* = 8.0

Both specimen have excellent fertility.

### The value of Flow cytometry in Fuchsia hybridization

In summary, I come to the following conclusions with respect to the value of flow cytometry in Fuchsia hybridization:

- 1. Flow cytometry is an excellent tool to check existing botanical Fuchsia material for its purity and to identify intraspecific variations such as an euploidy.
- 2. Flow cytometry is relatively simple, and an excellent tool, for establishing the origins of cultivars, particularly those formed from interspecific crosses. Possible deviations due to the attribution of the wrong parentage (think of self-pollination), or by the contribution of unreduced gametes, will then be clearly revealed.
- 3. Flow cytometry is an excellent tool to verify the results of (artificial) chromosome doubling.
- 4. Flow cytometry is an excellent tool for plausible explanations of the genomes of cultivars of (partly) unknown origin.

A warning should however also be made. Specifically for more complex crossings, flow cytometry could easily loose part of its value because of, eg., the occurrence of aneuploidy, the occurrence of recombinant chromosomes (by crossing-over in meiosis) resulting from interspecific crossings of species with very different 2C-value, and by a possible loss of DNA material in the formation of neopolyploids.

#### References and Remarks

- [1] J.Dolezel. J.Bartos, 2005; Plant DNA flow cytometry and estimation of nuclear genome size. Annals of Botany **95**: 99 – 110.
- [2] J.Greilhuber, J.Dolezel, M.Lysak, m.Bennet, 2005; The origin, evolution and proposed stabilization of the terms 'genome Size' and 'C-value' to describe nuclear DNA content. Annals of Botany **95**: 255 260.
- [3] The flow cytometry measurements have been performed by the Iribov BV company for the NKvF Hybridizers Group.
- [4] J.Greilhuber, 2005, Intraspecific variation in Genome Size in Angiosperms: Identifying its Existence; Annals of Botany 95: 91 98.

### New F. triphylla cultivar



Fuchsia 'Purcellian Elegancy'

### Farewell

Because of age and some health problems, Burgi and Rainer Klemm have decided to cease their activities in *Fuchsia* Hybridization.

They have compiled a beautiful overview of Burgi's Fuchsia introductions over the years, and have distributed this to their Fuchsia friends.

These cultivars are still available for purchase at the Gleichweit and the Pichler-Kobam nurseries (to be found on the internet).

We wish Burgi and Reiner all the best in the years to come!

#### Fuchsia 'Purcellian Elegancy'

Fuchsia 'Purcellian Elegancy' is the first pink F. triphylla to be released. This Fuchsia is a representative example of the first series of pale pink Fuchsias that has been produced in the programme 'In search for the white F. triphylla'.

Because the pink *F. triphylla* seedlings show a broad morphological variation as to the color of the flower and leaves, shape, growth and overwintering properties, additional introductions will most certainly follow in the coming years. These will all carry the prefix Purcellian', indicating that these cultivars are still *F. triphylla* species varieties.

The prefix is a tribute to the great British composer Henry Purcell, who has composed such wonderful music masterpieces.

'Purcellian Elegancy' has excellent fertility, both as the male and the female. It has good growth, however less vigorous than its male predecessor *F. triphylla* 'PB 7760#7'. If grown from autumn cuttings, without pinching, it

will start flowering early July. The flowering period extends some 6-8 weeks, and will restart in the autumn. Depending on the point of time cuttings are taken, and by pinching, the flowering period can be elongated over a longer period, which is attractive for hybridization purposes.

Overwintering properties of the 'Purcellian' series has still to be investigated more thoroughly. It is therefore recommended providing overwintering conditions similar to several more tender Triphyllas, i.e., applying a not too low temperature, preferably some 8°C or higher in the winter season. As a kind of insurance, autumn cuttings could be overwintered at higher temperatures.



Henry Purcell (1659-1695)

Photographs: Edwin Goulding

# Encliandras-Geographical Perspectives

By Edwin Goulding

#### **Abstract**

This article considers which Sections and Species of Fuchsia are closest geographically in the wild to Section Encliandra. It also asks whether they can be used to develop a range of Encliandra hybrids to create new international markets. It describes the options available and shows some of the work carried out so far.







F.fulgens



F.splendens



#### Species Involved Section Ellobium

- 1. F.decidua Mexico
- F.fulgens Mexico
- 3. F.splendens Mexico

All the species in this section come from Mexico, at the northernmost limit of the range of the Genus *Fuchsia*. They have some very significant features setting them apart, visually, from Section Encliandra and all the other species described here. They have long or moderately long tubes, often constricted near the ovary to avoid unnecessary

nectar loss. This means they can only be pollinated by birds with long bills and tongues or conceivably by a few insects with very long probosces. In terms of time and distance it is probable that pollen from Section Encliandra, developed for fertilisation in miniature flowers, would experience problems in fertilising such long blooms. But, they can be used as pollen bearers with more confidence of success.

Those species from this Section, that have been collected in the wild and tested for their chromosomal values, have been found to have Haploid counts of 11. *F. fulgens* and *F. decidua* occur frequently as epiphytes in the



F.cylindracea



F.encliandra ssp tetradactyla



F.microphylla ssp aprica

wild; this infers they survive mainly on atmospheric moisture like that found drifting through Cloud Forests. *F.splendens* is the most widely distributed, and variable in appearance. *F.decidua* and *F.splendens* seem to flower during the darkest months in East Anglia, given moderately adequate growing conditions. This is important in view of the fact that Encliandra flowering is at its best at the same time. The first two of those mentioned have swollen roots but this seems of no immediate significance to hybridists.

Reference: Breedlove et al, 1982.

#### Section Encliandra

- F.cylindracea ♀ Mexico
   F.parviflora was renamed; for details see
   Reference: Goulding (2002), p.128.
- F.encliandra ♂ Mexico
   One sub-species at the northern extremity, others all the way down to Nicaragua.
- F.microphylla ♀ Mexico
   Each sub-species occupies a different area down as far as Panama.
- 7. *F.obconica* Mexico In this country only.
- F. ravenii Mexico
   This species occupies a very limited area in Mexico.

#### 9. F.thymifolia – Mexico

F.thymifolia ssp. thymifolia is found in Mexico but F.thymifolia ssp. minimiflora is found at this country's lower end and into Guatemala.

All the species in this section come from Mexico, although their sub-species extend somewhat into Central America. They inhabit these northern limits more densely than other members of the Genus. Pollination in the wild is carried out by a variety of insects and birds, although it has been stated there is some specialisation of these within the Section. Their flower sizes are extremely small by comparison with most others in the Genus *Fuchsia*. Co-incidentally, the majority of miniature flowers occur at this northern extremity in the range, down







F.ravenii



F.paniculata (tetraploid by Colchicine treatment)

as far as Costa Rica. Some separation of the sexes is found within this section and many, especially single sex ones, are not found in cultivation within Europe.

All the species in this Section, that have been collected in the wild and tested, have been found to have a Haploid chromosome count of 11. (This could be disputed because F.microphylla was found to have 22 as well as 11.) Flowering occurs, sometimes spasmodically, throughout the year and can vary from plant to plant. Mia Goedman's criterion has been adopted here when separating F.encliandra from F.microphylla; the former having branchlets held at acute angles (30°) to the branches; the latter holds them at right angles (90°) to the larger branches. In fact, of course, all except F. ravenii, in which new shoots are juicier, have thin, wiry and shrubby growth. F.thymifolia has the stiffest, most upright and vigorous habit. It is worth noting, for the hybridist, that each berry produced by plants in this Section carries few seeds, perhaps only 1 to 5.



Note:- ¹ The exact status of many in this section, in cultivation, can be disputed. Observations of pollen fertility show percentages that appear much lower than could reasonably be expected. (Of course, pistillate blooms cannot be tested in this way.) Some plants also show variation in their flower form (i.e. perfect, pistillate and staminate) over time.



F.arborescens



F.thymifolia



F.jimenezii





F.triphylla

F.pringsheimii

#### Section Jimenezia

10. F.jimenezii – Costa Rica

The single species in this section comes from Costa Rica, at the southernmost end of the region in which the Sections and Species under consideration here are found. Several plants, claimed to be of this Species, are in cultivation but most do not carry blooms in terminal racemes or few branched panicles as described by Breedlove et al (1982). It is likely that pollination is via a number of different vectors in view of the small red blooms; white appears to be favoured by flies whereas bees seem to favour a wider range of colours, and birds frequent red, violet and orange flowers more commonly. Paul Berry (et al, 1982) lists this Species as having a Haploid count of 11 chromosomes.

The habit of growth resembles that of the encliandras but is stronger and stiffer than all except, perhaps, F.thymifolia. Like the encliandras it has a long flowering season although it sometimes rests intermittently for brief periods. It can be seen from the discussion so far and information still to come, that chromosomal variations are lacking throughout most of the sections being discussed; where tetraploidy does occur so do possibilities for hybridists to institute more radical changes in the appearance of offspring. In the absence of natural doubling, variation in pollen aperture numbers as seen under the microscope becomes a helpful adjunct. Since restrictions were placed on the availability and usage of Colchicine as an aid to chromosome doubling for amateur hybridists, no completely satisfactory alternative appears to be readily available. Vague talk exists about the value of Coffee and

some licenced chemical products. No technique for creating polyploids artificially has been written-up as a working protocol for use with *Fuchsias*.

Reference: Breedlove et al, 1982.

#### Section Schufia

- 11. F.arborescens Mexico
- 12. F.paniculata Costa Rica

The first striking feature about the two Species in this Section is that they grow quite a long way apart geographically, F.arborescens inhabiting the northernmost part of the range. Of the specimens that are in cultivation, this one is very difficult to identify and even harder to use in hybridising; this in view of the list of interspecific crosses made already using plants from Section Encliandra as given below. It seems much more likely that F.paniculata was used in this cross as it has been used with considerable success across a number of Sectional boundaries in the past. A number of variants of the latter Species are also in cultivation. Berry et al (1982) gives 11 again as the Haploid number of chromosomes for each of these species.

Geographically, it seems strange that Fuchsia is not listed as occurring more widely throughout Nicaragua, Honduras, El Salvador or Guatemala. Just whether this is due to a lack of suitable environments, especially cloud forests, is uncertain. Growth in the Section Schufia can be almost tree-like and this could impede the development of small pot grown hybrids. The Section, like all of



Seedling (a.5)

Seedlings a.1-a.9 have been derived from the crossing (F.paniculata x F.jimenezii) x [F.ravenii x (F.microphylla ssp. aprica x F.lycioides)].



Seedling (a.1)



Seedling (a.2)



Seedling (a.3)



Seedling (a.4)



Seedling (a.6)

the others from Central America under consideration here, is not reliably frost hardy. However, blooms carried in dense terminal panicles have a distinct visual appeal even if they are only of a small individual size. This is evident in Lilac, another popular garden shrub.

Of course, the ideal for hybridists would be to increase the flower sizes. Colours also need to become much more varied. Bicolours and doubles are highly desirable as are much larger petals; quality plus quantity. Crossings between the various Sections in the region should turn these possibilities into realities.

Reference: Breedlove et al, 1982.

#### Section Fuchsia

- 13. F.pringsheimii Hispaniola, Haiti and the Dominican Republic
- 14. *F.triphylla* Hispaniola, Haiti and the Dominican Republic

There remains a further possibility to consider, the use of Fuchsias currently listed as being from Section Fuchsia that exist naturally in Hispaniola, an island in the West Indies. Certainly, the first of these carries several features more generally associated with Section Encliandra. Their advantages include naturally occurring tetraploidy; each listed (Berry et al, 1982, p.36) as having Haploid = 22 chromosomes, although the latter is also shown as carrying 44. Pollen studies show wide variation in aperture numbers in specimens collected from F. triphylla. A wide range of variation also exists in the visual appearance of plants of the two species, now in cultivation.

Reference: Berry, 1982.

# Interspecific Crossings with Section Encliandra (see notes 2 & 3)

F.cylindracea x F.paniculata

x F.splendens

- F.encliandra x F.fulgens
  - x F.paniculata
- F.michoacanensis<sup>2</sup> x F.cordifolia<sup>3</sup>
  - x F.obconica
  - x F.paniculata
  - F.obconica x F.arborescens
    - x F.fulgens
    - x F.splendens
    - x F.splendens
  - F.splendens x F.microphylla ssp. aprica
    - x F.ravenii

#### Notes:

F.ravenii

- <sup>2</sup> No such species is described by Breedlove (1969) or has been authenticated since.
- <sup>3</sup> F.cordifolia has been classified as being just one of many F.splendens variants by Breedlove et al (1982).

#### Discussion

#### Flower form:

Pistillate *Fuchsia* flowers are really too small for the general pot plant markets and this is also true of staminate ones. The former have one slight advantage for the hybridist, however, in that they seem more willing to accept pollen from a wider range of other species and cultivars than is to be expected from plants with perfect flowers. Their seed growing ability also appears to be slightly better. Now, while this might be advantageous in the early stages of Encliandra development the continuous production of large numbers of single sex hybrids in the later stages of a programme would be a major disadvantage.

#### Availability of stock:

One of the key factors influencing the formation of hybridising programmes such as the one under discussion is whether the plants chosen as being optimal are actually available. Sometimes they can be obtained through friendly contacts. Less commonly they can be bought from specialist nurseries. The necessity for supporting the latter has still not been recognised by *Fuchsia* societies who appear to think profit and greed go hand in hand. In fact species *Fuchsias* are



Seedling (a.7)



Seedling (a.8)



Seedling (a.9)

Seedlings a.10 and a.11 have been derived from the crossing (F.paniculata x F.jimenezii)



Seedling (a.10)



Seedling (a.11)

highly unlikely to pay their way in a commercial setting; they require more attention than hybrids, produce fewer cuttings and demand for them is very small and highly spasmodic.

#### Adaptability:

One key concept in the theory of Evolution is that variability is an essential ingredient in the fight for survival. *Fuchsia* growers should be aware that this is as true of the market place for plants as it is of their dwindling natural habitats. Being ideally suited to niche conditions can be a severe disadvantage if those conditions change (as any Dodo would tell you if it could). Co-dependent plants are also vulnerable because they are as reliant upon their pollinators as they are on other environmental factors for their survival.

#### Outbreeding:

Increased genetic variability increases the health and vigour of stock. Inbreeding weakens plants and animals alike. The more inbreeding that occurs the more likelihood there is of genetic weaknesses and deformities occurring. In this context the idea of this article is that several Sections growing in relatively close Geographical proximity can strengthen the genetic base from which development can take place. Furthermore, these sections share many features that should prove helpful to *Fuchsia* hybridists.

#### Seasonality of Flowering:

The average hybridist will realise that many crosses have proved impossible because seed bearing and pollinating parents refuse to bloom at an opportune moment, together.

The market for today's depleted range of wholesale cuttings is at best around three months, at the start of each growing season. In spite of this, stock plants from which these cuttings are taken must be grown for twelve months each year with all the associated costs in such things as labour. Shows and competitions further restrict the flowering season so that many so-called enthusiasts neglect their plants after the early summer and, if able to, go away on holiday.

This is where Encliandras are so promising. They have naturally long flowering periods but are frequently quite happy as day lengths shorten and light intensity weakens. The size, quantity and quality of blooms improve. They are also quite content with a wider range of temperatures than hybrids developed from Section Quelusia. The average household in the United Kingdom will have temperatures around 20° Celcius; even the majority of indoor fluctuations are within acceptable limits for Encliandras. Relatively low light levels are also more easily adapted to than would be the case for plants from any other South American *Fuchsia*. There is scope for the ideal conservatory flowering pot plant to be produced.

#### Work in progress and previous results:

The pictures of seedlings a.1-a.14 show how some early work is progressing. Most show little variation in flower type (therefore, these are not shown in the photographs), whereas foliage shows clear genetic differences.

The first group of photographs (a.1 to a.9) shows seedlings derived from (F.paniculata x F.jimenezii) x [F.ravenii x (F.microphylla ssp. aprica x F.lycioides)]. The latter species is a tetraploid that provided early success in crossing the interspecific boundaries with Encliandras. It is not featured in detail because it comes from Chile and is prone to several of the more serious problems currently besetting the Genus.

The remaining photographs of recent seedlings show some of those raised from different crosses. The first two (a.10 & a.11) are from (F.paniculata x F.jimenezii) as the seed parent and a very large un-released double acting as the pollinator. A further photograph (a.12) shows a plant with the following parentage, (Obcylin x F. ravenii) x (Obcylin x F. ravenii). The penultimate picture (a.13) is of (Obcylin x F.ravenii) x [Obcylin x (F.paniculata x F.jimenezii)]. We could also talk about crosses made using plants from Section Hemsleyella as pollinators and as illustrated in the final photograph (a.14), but all of that is another story for some other time.

The photographs in the picture gallery on pages 15-16 provide an impression of the beauty of crossings combining more elements from the region within the overall concept of Encliandra.



Seedling (a.12)

Seedling a.12 has been derived from the crossing (Obcylin x *F.ravenii*) x (Obcylin x *F.ravenii*)



Seedling (a.13)

Seedling a.13 has been derived from the crossing (Obcylin x *F.ravenii*) x [Obcylin x (*F.paniculata* x *F.jimenezii*)].



Seedling (a.14)

Seedling a.14 has been derived from a crossing using a plant from the Section Hemsleyana.

#### Conclusion

It is all too easy to stay with the status quo. Change requires effort, time, and an adventure of faith. The ideas discussed are no longer impossible dreams.

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**'Katinka'** Mia Goedman-Frankema - 1993

'Katinka' originated from a cross between F. ravenii x F. microphylla ssp. microphylla. Fertility between such crosses is often non-existent or much reduced. 'Katinka' self-set seeds.



'Neapolitan' D. Clark -1984

This introduction has the characteristics of an interspecific plant except that it is highly fertile and has variable progeny. Its parentage has not been published.



'Neapolitan' x 'Neapolitan' (1985)



'Neapolitan' x 'Neapolitan' (1985)



'Neapolitan' x 'Neapolitan' (1985)



**'Lechlade Tinkerbell'** Wright -1983 F. arborescens x F. thymifolia ssp. thymifolia



**'Rijs 2001'**De Boer - 2001

F. obconica x F. splendens



**'Small Pipes'** De Graaff - 1985 F. paniculata x F. triphylla



**'Thumbelina'** Goulding Fuchsias - 1995 'Lechlade Tinkerbell' x 'Wapenveld's Bloei'



**'Wapenveld's Bloei'** Kamphuis - 1991 Baby Chang' x *F. arborescens* 

# New fuchsias from Mario de Cooker (NL)



Fuchsia 'Icicle'

#### Fuchsia 'Icicle'

Fuchsia 'Icicle' (De Cooker, 2015) originates from the crossing {['Winter Charm' x ('Delicate White' x 'Stanley Cash')] x ('Göttingen' x 'Our Ted')} x (Papy René x Papy René).

F. 'Icicle' is a self branching, vigorous and floriferous cultivar. Flowers are produced in large racemes which make a massive display. It is best grown as a semi trailing bush in a somewhat sheltered position in filtered light, although it tolerates high temperatures and some sun. Overwintering is without any problems.

The cultivar's name: 'Icicle' reflects the shape and colour of its flowers, hanging down like icicles from the gutter on a bright winter's day.



F. 'Icicle' raised from autumn cuttings (July 2015)



Fuchsia 'Candy Ball'

#### Fuchsia 'Candy Ball'

Fuchsia 'Candy Ball' (De Cooker, 2015) is a Triphylla hybrid cultivar originating from the crossing F. triphylla 'HvdP' x ('Playboy x ?'.

It is best grown as a bush in a somewhat sheltered position, in any case in filtered light at high temperatures during summer.

Overwintering is without any problems.

The cultivar's name 'Candy Ball' reflects the bright shining pink colour of the flowers, in combination with the tube being shaped like a small round to oval ball.



F. 'Candy Ball' grown from an unpinched autumn cutting (July 2015)

# Analysis of the composition of the genome of Fuchsia 'Winter Charm'.

By Mario de Cooker

#### Summary

The composition of the genome of the fertile *Fuchsia* 'Winter Charm' is very likely to be allotriploid JIM (= *F. juntasensis* + *F. inflata* + *F. magdalenae*).

#### **Analysis**

Fuchsia 'Winter Charm' (a winter flowering, vigorous, trailing Fuchsia cultivar) was created through the following steps:

#### First step:

F.inflata x F.juntasensis) = I 90-01. This cross was made in 1989 by the Dutch hybridizer Jan van den Bergh, and is still available for hybridization purposes. Fuchsia I 90-01 clearly has characteristics of both F.inflata (n=22) and F.juntasensis (n=44). From flow cytometry measurements for I 90-01 the 2C-value amounts to 6.6. The 2C-value to be expected for this presumably triploid seedling with a genome composition of JJI, is 2C = 2 × 1.6 +3.4 = 6.6. There is excellent agreement between these two values [1].

I 90-01 appears moderately fertile both as a father and as a mother. Because I 90-01 is a triploid, very different gametes can be formed because each gamete can get copies of each chromosome in different combinations. In case of the hypothetical situation that the specimen would have, e.g., only one set of homeologous JJI chromosomes, gametes with chromosome composition J, I, JJ, JI and JJI could probably be produced. In reality, the situation will be far more complex. The number of possible combinations of chromosomes increases rapidly as the number of chromosomes increases. Therefore, the number of balanced gametes drops appreciably for genomes with larger numbers of chromosomes. Preferences and frequencies for pairing of specific combinations are not known, so numerous combinations could be produced, of which a substantial number will be aneuploid.

#### Second step:

This cross was carried out: I 90-01 x F.magdalenae = N 99-18. The cultivar N 99-18 is moderately fertile and was introduced as Fuchsia 'Winter Charm' in 2011.

By the appearance of N 99-18 (see photo), we may assume that this cultivar, as a result of the crossing described, contains at least both *F.juntasensis* as well as *F.magdalenae* chromosomes. The measured 2C-value is 6.4. This corresponds well with the genomic composition JJMM, of which the 2C-value is  $2 \times 1.6 + 2 \times 1.5 = 6.2$ . All of this seems a plausible assumption, as also a certain degree of aneuploidy might be present which will cause some deviation.

#### However, further information shows that a different composition of the genome is more likely.

In our analysis we can also add as pieces of information that:

- ◆ The shape of the berries of 'Winter Charm' (N 99-18) resembles that of *F.inflata* [2]. See also the photo on p.19 of berries from *F.inflata* on Henk Hoefakker's website [3].
- From the crossing N 99-18 x *F. fulgens*, seedling N 14-17 resulted.

The appearance of this *Fuchsia*, N 14-17 (see photo on p.20), suggests that in its female parent (which is N 99-18), *F.inflata* is obviously still present. At this point the influence of *F.juntasensis* seems to have totally disappeared. For comparison, the appearance resembles that of *F.* 'Treslong' = *F.magdalenae* x *F.inflata*. This *Fuchsia* seedling, N 14-17, has unfortunately not survived the winter, so we cannot measure its 2C-value. If desired, the crossing can of course always be repeated.



Seedling I 90-01



Fuchsia 'Winter Joy'



Fuchsia N 99-18 = 'Winter Charm'



Berry F. juntasensis



Berry F. inflata



Berry seedling I 90-01



Berry F. Winter Joy'



Berry F. 'Winter Charm'



Seedling N 14-17

If we assume that *F.inflata* is indeed still present in N 99-18, then at a 1C-value of 3.4 for *F.inflata* already half of the 2C genome of N 99-18 is provided by *F.inflata*. Given the crossing from which N 99-18 has originated, and its appearance, N 99-18 must also contain *F.juntasensis* and *F.magdalenae*.

All in all, hardly any freedom then exists anymore for making choices as to the genome of N 99-18. From all this it follows that, for N 99-18, a very plausible genome is JIM, with a 2C-value of 1.6 + 3.4 + 1.5 = 6.5. Other combinations are much less likely if we use the boundary conditions set by the crossings made and the phenotype. The 2C-value of 6.5 corresponds very well with the measured value of 6.4.

N 99-18 being fertile, this triploid cultivar could form viable gametes containing J, M, and I, and all kinds of combinations of J, M and I, so, gametes of very different compositions. If we accept that in the N 99-18 genome JIM is indeed correct, then *F.magdalenae* must have contributed an M gamete during the crossing. The literature shows that in crossings with an autotetraploid specimen this is quite possible [4]. See also the text box.

From I 90-01 emerged 'Winter Charm',

Winter Joy' and 'Winter Hymn'.

'Winter Joy' is a self-pollination of
I 90-01 and resembles it. 'Winter
Hymn' is the result of the crossing
I 90-01 x [('Gottingen' x 'Our Ted') x
('Gottingen' x 'Our Ted')]; this crossing
is probably too complex for a currently
meaningful analysis to be made by flow
cytometry measurements.

#### In conclusion:

The combination of flow cytometry and the phenotype of the seedlings and berries originating from crossings with I 90-01 allows us to distil the following information with respect to the genomic composition and the genesis of N 99-18 = F. Winter Charm':

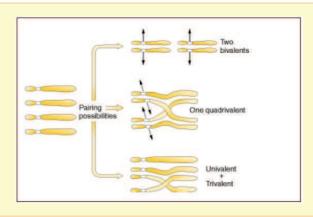
- The fertile *Fuchsia* 'Winter Charm' is very likely to have the triploid genome

composition of JIM;

- The autotetraploid *F.magdalenae* has, in the formation of 'Winter Charm', contributed an M gamete having one set of chromosomes.

#### References and remarks

- [1] The flow cytometry measurements have been performed by Iribov BV for the NKvF Hybridizers Group.
- [2] From the cultivars I 90-01 and 'Winter Joy' it can be seen that the berry is longer than that of *F.juntasensis*, its morphology being influenced also by *F.inflata*. The light yellow / orange color of *F.juntasensis* and *F. inflata* is maintained. In 'Winter Charm' *F.inflata* influence is shown in the shape of the berry and its length (3-4 cm); but the color has become darker. Apparently, the influence of *F.magdalenae* is evident by the dark purple colour inherited from the *F.magdalenae* berry.
- [3] Mr. Henk Hoefakker's website: http://www.hoefakkerfuchsia.nl/
- [4] A.Cisneros, N.Tel-Zur, Evolution of Interspecific-Interploid Hybrids (F1) and Back Crosses (BC1) in Hylocereus Species (Cactaceae); In: Meiosis – Molecular Mechanisms and Cytogenetic Diversity; Edited by Dr. Andrew Swan; In Tech, 2012, ISBN 978-953-51-0118-5.
- [5] Besides the complexity of the crossings, also the mingling of genomes during meiosis, forming recombinant chromosomes, will undoubtedly muddle the conclusions.



A mating at meiosis in the form of a univalent and a trivalent is common in autopolyploids [4]. Unbalanced gametes could then be produced.

F.magdalenae is an autotetraploid Fuchsia species.





Delphobe: "I wish I could spend my holidays in the garden with the Fuchsias"

### Contents of the next issue

The next issue is scheduled for the end of December 2015.

# First steps in making Triphyllas with brown/bronze/purple foliage.

Brown/bronze/purple foliage is an attractive feature of many plant varieties. First steps have been made in making Triphyllas with real brown-purple foliage. But a lot of work has still to be done.



#### Surprising effects in fuchsia hybridization.

Several triploid and pentaploid *Fuchsia* varieties prove to have excellent fertility. Understanding the results of crossings with such fuchsias is however not a straightforward exercise. Large variations in genomic composition of gametes occur, and even gene silencing seems to play a role.

#### Sowing Fuchsias: The System.

Any system is only as good as the parts on which it relies for its operation. This is as true of sowing *Fuchsia* seeds and of raising our own seedlings as it is of any more complex industrial process. This article by Mr. Edwin Goulding is about one such system.

# So stay connected!

Your contribution to the contents of The Fuchsia Breeders Initiative is highly appreciated. Contributions for the next issue should be made available at the latest on 1 December 2015.

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