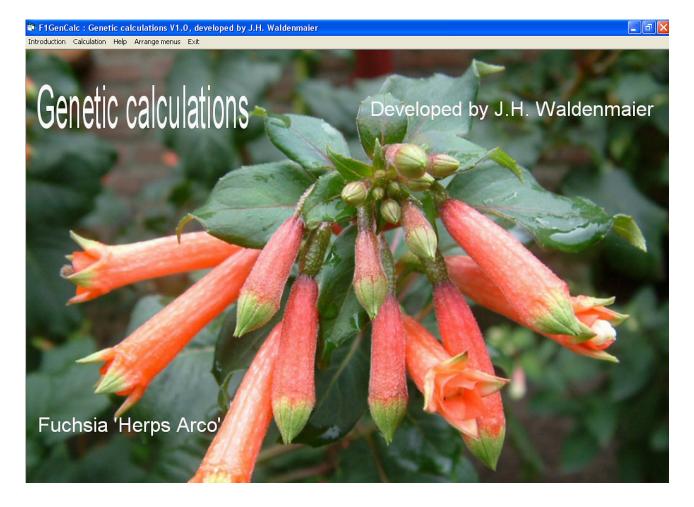
# **F1GenCalc**, a Visual Basic Program for calculations of F1 geno- and phenotypes given the parental genotype(s) and mode(s) of inheritance.

- Suited for polyploidy up to octoploids
- Processes maximal 5 loci simultaneously
- Handles Linkage, FDR/SDR and Double Reduction



## <u>Contents</u>

F1GenCalc	3
Introduction	
Procedures	
Flowdiagram	9
Allel specification	11
Linkage	
Division restitution	
Double reduction	17
Fuchsia characteristics analysed, with examples using F1GenCalc	20
Fuchsia flower colors.	20
Fuchsia flower type (single, semidouble, double)	

## F1GenCalc

#### Introduction

Given the parent(s) genotype this program calculates:

- gametic genotype distribution parent(s)
- > F1 genotype distribution
- > F1 phenotype distribution

Developed by J.H. Waldenmaier, St Sebastianusstraat 52, 5373AE Herpen, tel. 0486-414504 e-Mail: henkwaldenmaier@home.nl

This Visual Basic program can handle polyploid plants (up to octoploids) with multiple loci (up to 5). Linkage, double reduction and meiotic division restitution is taken into account.

The genotype of a parent may contain as many different genes per locus as there are alleles.

Division restitution can be specified as a percentage for FDR (First Division Restitution) and for SDR (Second Division Restitution).

DR (Double Reduction) can be specified for each locus as a percentage of the total DR possible for that locus.

Linkage can be specified as a percentage for every combination of two different loci.

The following ways of inheritance are supported:

Quantitative ( = additif) characteristics
These are characteristics that can be added together resulting in a final number that reflects the final phenotype (e.g. flowertube length). In case of polyploidy most characteristics can be best addressed to as quantitative in nature in order to cope with the number of alleles >2.

> Qualitative characteristics These characteristics are based on the present/absent principle in the phenotypes, taking into account the possibility of intermediair inheritance and epi/hypostatic inheritance. Quantitative and qualitative ways may be mixed in one genotype.

All distributions (gamete genotype, F1-genotype and F1-phenotype) are expressed in frequencies varying between 0 and 1.

#### **Procedures**

Starting point for the calculation of the gametic distribution always is the **complete theoretical distribution** for the ploidy level of the parent, regardless the other specifications made for the parent genotype. The theoretical gamete distribution is calculated, sorted and filed on the disk. This <u>theoretical gamete distribution</u> always concerns <u>one locus</u>.

Next, data-reduction is done. Gamete-genotypes <u>not applicable</u> (e.g. with DR while DR% is specified as zero) will be excluded. The theoretical genotype for each gamete will be replaced by the genes as specified in the parent. Frequencies of similar gametes will be <u>summed</u>. A cartesian product of the gametes per locus is calculated to get the <u>total genotype</u> of all gametes. Again gametes of the same genotype are <u>summed</u>. If <u>linkage</u> is specified for some loci, a <u>recalculation</u> of the obtained gamete-frequencies is performed.

Having established the gamete-distributions of both parents in this way, a cartesian product of these distributions gives the F1-genotype distribution. Again identical genotypes are <u>summed</u>.

Conversion of these **genotypes** into **phenotypes** is one according to the specified phenotype data. Identical phenotypes are <u>summed</u>.

There are two ways characteristics can be specified:

- Quantitative
- Qualitative

Or a combination of both.

#### > QUANTITATIVE (additif) characteristics

This characteristic can consist out of one or more loci. Each gen-allel contributes to the final phenotype. This contribution is calculated using the following formula:

> Additif % divided by the number of genes for this character over all loci concerned.

Moreover interaction between loci is possible.

#### Example

Let's say there are two loci for a quantitative characteristic. Locus 1 has the genes A, A1 and a. Locus 2 has genes B and b.

Additif % is specified as follows:

Α	100
A1	80
Α	20
В	60
b	0

This will result in the following distribution given a genotype of AABb:

	Total	65
b	0/4	0
В	60/4	15
Α	100/4	25
Α	100/4	25

<u>Interaction</u> between loci takes place if interaction is specified as a factor (between 1.0 and 2.0) and if on both loci a gene with an additif % of 50 or more is present.

In the above example let's assume the interaction factor between locus 1 and locus 2 was specified as 1.5. Because A and B are both present in the genotype (and both have an additif percentage greater than 50%) the final phenotype will be  $65 \times 1.5 = 97.5$ .

#### > QUALITATIVE characteristic

A qualitative characteristic may consist out of one or more loci. For <u>each gen-allel a description</u>, <u>dominance-order and expression %</u> is specified. Moreover, a combination of two phenotypic outcomes can be overruled by a specified new phenotypic description, thus allowing for intermediair

inheritance (outcome 1 + outcome 2 = outcome 3) or epi/hypostatic inheritance (outcome 1 + outcome 2 = outcome 1 or outcome 2).

Phenotypic outcome ( = gene description) is taken when the number of the specific gen within the locus >= the concerning expression percentage. If no expression % is reached for any phenotypic outcome the outcome for the most dominant gene present (that means with the lowest dominance order number) will be taken.

When two or more outcomes are established replacement is done for outcome-combinations specified as intermediair and/or epi/hypostatic. If there are no relevant outcome-combinations, the outcomes will be replaced by the outcome for the most dominant gene.

#### Example

Let's say there are two loci. Locus 1 with genes A, A1 and a. Locus 2 with genes B, B1 and b.

#### The gene-descriptions are:

- $\triangleright$  A white
- orange
- red
- blue
- yellow ▶ B1
- > b black

#### As <u>combination</u> the following is specified:

> white + yellow = salmon (intermediair)

#### Dominance order:

- $\triangleright$  A
- > A1

2

- ▶ B1 4
- ▶ b

#### Expression %:

- 10
- 65
- 80
- ▶ B 75
- ▶ B1 60
- **>** b 70

## The following table gives genotypes together with the phenotype:

Geno	otype			Phenotype
Α	α	В	b	White
				(because of A)
Α	A1	B1		Salmon
	B1			(because of A and B1 = intermediair)
а	α	B1		yellow
	B1			(because of a and B1, with B1 as most dominant)
<b>A</b> 1	α	B1	Ь	Orange
				(because A1 is the most dominant)

## Flowdiagram

- Specify target
  - P gamete distribution
  - F1-genotype (includes Parents gamete distributions)
  - F1-phenotype (includes P-gamete distributions and F1-genotype)
- Input parent(s) genotype
- If specified: Input phenotypic way of inheritance
- For every parent:
  - get parent gamete distribution
  - Sort
  - Reduce gamet distribution according to specifications of parent-genotype input (including Double reduction)
  - Assign parent-genotype genes to gamet-genotype (unique codes)
  - Get cartesian product for all loci
  - Sort
  - Sum identical gamete genotypes
  - Is linkage specified?
    - ♦ No
    - Yes
      - > Classify each gamete on Yes or No crossing over
      - > Recalculate gamete frequency
- **F1-genotype** required?
  - No

- Yes
  - Get cartesian product of gamete distribution of both parents
  - ♦ Sort F1-genotype distribution
  - ♦ Sum identical F1-genotypes
- **F1-phenotype** required?
  - No
  - Yes
    - ◆ Convert F1-genotypes into F1-phenotypes
    - ♦ Sort F1-phenotypes
    - ♦ Sum identical F1-phenotypes

### Allel specification

By input a letter code is assigned to each specified locus.

For each locus there are as many alleles as specified by the ploidy of the parent, eg 4 in case of tetraploidy.

For each allel a locus-code must be specified. In general is the capital character used for the most dominant allel-version, and the low-case character for the most recessive allel-version. Intermediate codes (if those versions exist for the locus) can be formed using <u>the capital or lower case combined</u> <u>with one letter or number</u>. Note the maximum length of specified allel-codes is 2.

#### Example

Tetraploid parent, 2 loci, one for flower color= code A, the second for growing type = B. Possible alleles for flowercolor:

- > A purple
- > A1 red
- > A2 pink
- > aorange

#### Possible alleles for growing type:

- > B up-growing
- > B1 semi-hanging
- ▶ B hanging

#### Genotype parent (eq A A2 a a B1 b B b) could be specified as follows:

	Gen A-locus	Gen B-locus
Allel 1	Α	B1
Allel 2	A2	Ь
Allel 3	a	В
Allel 4	a	Ь

Note that locus A and locus B may or may not be located on the same chromosome. If they do, there might be crossing-over. In that case it is of importance if the loci on the alleles are in coupling or in repulsion.

In coupling mean that the dominant version of both loci is on the same allel, in repulsion is the opposite (on the same allel one of the loci has the dominant version, the other locus the recessive version).

To make the allel-codes unique, the program adds a relative numer for each locus in front of the inputted allel-code, starting with 1 for the first locus.

#### In the example:

	Α	->	1 <i>A</i>
>	A2	->	1A2
>	α	->	1a
>	В	->	2B
>	B1	->	2B1
	h		2h

## Linkage

Entered and stored as a percentage for each loci-pair.

This percentage, called linkage %, presents a measure of the number of genetypically detectable crossing over gametes in the F1-generation, and is therefore an indication of the degree of linkage.

So, for 2 loci, a percentage is present for:

➤ Locus 1 - locus 2

and for 5 loci, a percentage is present for:

- Locus 1 locus 2
- ➤ Locus 1 locus 3
- Locus 1 locus 4
- Locus 1 locus 5
- Locus 2 locus 3
- Locus 2 locus 4
- Locus 2 locus 5
- Locus 3 locus 4
- Locus 3 locus 5
- Locus 4 locus 5

Do not confuse this percentage with the linkage % as used in theoretically linkage calculations, where it is strictly related to the distance between the loci (recombination fractions). The linkage % as used in the underlying system provides the factor by which gametes with genotypically detectable crossing-over should be reduced in frequency (and consequently gametes without detectable crossing-over should be increased).

This method is chosen for mainly 2 reasons:

> Even if linkage is established using the distance between the loci chiasma interference as well as chromatid interference will cause unpredictable results.

> Practically the calculation of gamete-frequencies including double reduction is nearly impossible using loci-distances and the corresponding permutation procedures. The pioneer theoretical paper on this topic is from Fisher (Fisher, 1947, The theory of linkage in polysomic inheritance, Phil. Trans., B, 233, 55 87). Also Bailey (N.T.J. Bailey, 1961, Introduction to the Mathematical Theory of Genetic Linkage, Oxford, at the Clarendon press, page 113) mentiones this problem:

"So far there is no theoretical basis for predicting the frequency of any given mode of gamete formation in terms of, for example, the recombination fraction between the two loci and the two double reduction parameters".

For this reason the method presented here was developed. Of course there is a relation between both linkage percentages.

#### Procedure

Establish the gamete distribution as if the loci were present on different chromosomes. This corresponds with the case of loci on the same chromosome, but with a distance between them so large, that crossing over results in a gamete-distribution conform not linked loci.

Next determine for each gamete and for each loci-pair involved, if its genotype contains at least one gencombination, not present on the alleles of the parent ( = detectable crossing over). Sum the frequencies of these gametes per loci-pair.

Calculate the crossing over factor:

Factor = sum - ((linkage %) / 100% x sum)

Continue with recalculating the frequencies of all the gametes, using the following formula:

If crossing over in the loci-pair: frequency x (factor/sum).

If no crossing over in the loci-pair: frquency  $\times$  ((1-factor)/(1-sum)).

Do so for every loci-pair.

#### Example

Parent diploid, 2 loci, linkage % between locus 1 and locus 2 is 60%, no double reduction, no FDR, no SDR.

Genotype parent:

locus	Allel 1	Allel 2
1	Α	a
2	В	Ь

This results in the following gamete distribution:

		<del>-</del>
gamete	Frequency	Detectable crossing over (Y/N)
AB	0.25	N
Ab	0.25	У
αB	0.25	У
ab	0.25	N

Sum of frequencies of gametes with detectable crossing over:

(Ab) 0.25 + (aB) 0.25 = 0.5

Crossing-over factor:  $0.5 - (60/100) \times 0.5 = 0.5 - 0.3 = 0.2$ 

Recalculation of gamete frequencies:

gamete	Old	Crossing	Multiply	New
	frequency	over	with	frequency
AB	0.25	N	(1-0.2)/(1-0.5)	0.4
Ab	0.25	У	0.2/0.5	0.1
αВ	0.25	У	0.2/0.5	0.1
ab	0.25	N	(1-0.2)/(1-0.5)	0.4
Total	1.0		Total	1.0

#### Division restitution

Meiotic division restitution means lack of one of the two reduction divisions during meiosis. Because of this the number of chromosomes in the resulting gamete is exactly the same number as that from the parent in stead of half of it.

Because there are two reduction devisions during meiosis, there are two possibilities:

- First Division Restitution (FDR). Because no chiasma's are formed, no crossing overs are possible.
- > Second Division Restitution (SDR). Because chiasma's may be formed in the first reduction division, crossing overs are possible.

Triploids are mostly infertile, because reduction in two parts is not possible. In some cases FDR (or SDR) is present in triploids, and those gametes are mostly the only fertile ones formed (FDR=100%).

Division restitution can be triggered by <u>high temperatures</u> during meiosis, but mostly it is (partly) inherited.

#### Double reduction

The cromosomes at the beginning of meiosis are really paired structures, each one consisting of <a href="two-chromatids">two-chromatids</a>. Crossing over is thus a process that may involve all four chromatids of the two paired chromosomes. The process of gamete formation is equivalent to the selection of two chromatids, which then assume the role of chromosomes.

The passing of both chromatids of a single chromosome to the same gamete is called Double Reduction (DR). The amount of DR has to be determined from observed F1-distribution data (based on the frequency of phenotypically detectable fully recessive individuals from crosses where total recessives are impossible to occur without the DR-phenomenon).

In literature generally alpha is used for the percentage of DR where gamet-ploidy is 2n. In triploid gametes beta is used (2 out of the 3 chromatids can be formed by one DR-event). In tetraploid gametes two parameters are suggested (one for gametes with one event of DR, the other in case all 4 chromosomes are formed by two events of DR). And so on.

Practically, it is very hard to work with this modell. For example, imagine the difficulties to establish the different DR percentages based on phenotypic outcomes from different crosses in case of high polyploidy.

For this reason a new calculation procedure was developed.

#### **Procedure**

It seems likely that the frequency of <u>DR is a constant for any given locus</u>, depending on <u>its distance from the centromere</u> (N.T.J. Bailey, Introduction to the mathematical theory of genetic linkage, Oxford, at the Clarendon Press, 1961, page 106).

The amount of DR in a particular case can never exceed a specific maximum. For example if we backcross a triplex AAAa individual to the recessive aaaa the proportion of recessives amongst the offspring is  $0.25 \times \text{alpha}$ . Such a mating gives a direct estimate of alpha, which would be equal to 1/7 if chromatid segragation were completely at random for we should have  $\frac{1}{4} \times \text{alpha} = 1/28$ . One would expect to observe frequencies that were rather less than this maximum at random segregation, since there are cytological grounds for supposing that in reality conditions are intermediate between random segregation of the

<u>chromatids and the diploid type of segregation</u> (C.D. Darlington, 1931, Meiosis in diploid and tetraploid Primula sinensis, Ibid, 24, 65-96).

In the developed procedure the DR-formation in the gametic genotype follows exactly the procedure as described in "Introduction to the mathematical theory of genetic linkage", N.T.J. Bailey, chapter 7 polysomic inheritance". Therefore, this method will not be explained further here.

The <u>new approach</u> concerns the calculation of the frequencies of DR-gametes in the P1-gamete distribution.

A formula was developed to calculate the maximal possible amount of DR in all relevant cases (ploidy-level of the parent, ploidy level of the gamete, number of DR-events in the same gametic genotype). As input a percentage (per locus) is requested (called DR %), that gives an estimation between total random segregation (100% DR) and total diploid segregation (0% DR), taking into account the distance from the locus to the centromere. This percentage is related to the maximal DR possible.

So, with this procedure, different DR% for 1, 2, 3 etc. DR-events are avoided. Moreover a formula was developed to calculate the total number ( = divisor) for the theoretical gametes for the specific gametegenotype including the different DR-cases, in order to be able to calculate gamete frequencies.

These two formulas are:

> Maximal possible DR

#### 

where DR = Maximal DR frequency

Np = Number of alleles in parent (eg tetraploid=4)

Ng = Number of alleles in gamete (eg diploid=2)

Ndr = Number of DR-pairs in gamete

## > Divisor

## Fuchsia characteristics analysed, with examples using F1GenCalc

#### Fuchsia flower colors

Flower colors in Fuchsia's have as main determinants anthocyanin pigments, and vary from orange via pink/red to lilac/blue/purple. Lack of flavonol pigments results in the absence of yellow flowers. Silence of the anthocyanin biosynthesis pathway (silence of CHS, DFR or F3H genes) results in white flower varieties. See also page pigmentanalyse: http://members.home.nl/henkwaldenmaier/pigmentanalyse.htm

The main chemical process in forming anthocyanins from flavonoids is hydroxylation, resulting in the anthocyanin-groups Pelargonidin (PG, no hydrolysis, orange to red), Cyanidin (CY, hydrolysis on R1, red to magenta) and Delphinidin (DP, hydrolysis on both R1 and R2, magenta to purple).

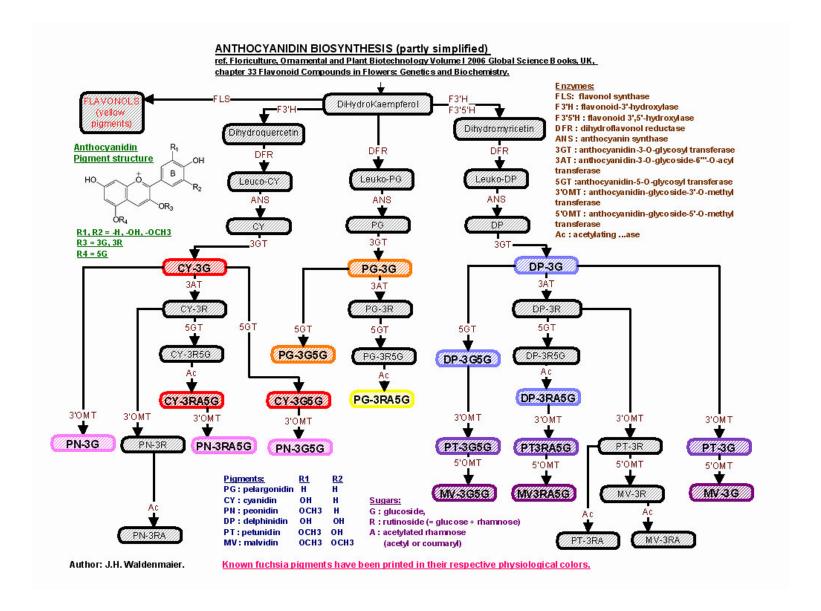
The effects of methylation, glucosidation (on R4) and co-pigmentation is mainly intensification of the colors (pink into red, lilac into blue/purple, brighter colors). For that reason (and to simplify the model) in first instance only hydroxylation was included in the genetic calculation procedure.

The vacuole pH also can alter the color spectrum, but as long as the acidity of the vacuole fluid hardly can be altered by genetic and/or external circumstances it is in first instance ignored in the F1GenCalc calculation.

On the next page the (simplified) anthocyanidin biosynthesis is given, as far as it is relevant for the pigments found in Fuchsia flowers. In general there is an ascending order in hydrolysis-dominance from PG (less dominant) to CY (dominant) and DP (most dominant).

F1GenCalc can cope with quantitative and qualitative phenotype translation. When dealing with diploids mostly qualitative inheritance will be followed (100% recessive, intermediair (if present), dominant (hetero and/or homozygotic). In case of polyploidy the increased number of alleles favours nearly always the quantitative approach. This means that the phenotypic outcome results from F1GenCalc specifies frequencies for different quantitative classes of the characteristics examined. Translation of these classes into actual phenotypic appearance have to be done additionally.

[ For explanation of R1, R2, R4, CH5, DFR, F3H see next page ].



Link to the referenced literature: <a href="http://www.danforthcenter.org/yu/pdf/e-flower-2006.pdf">http://www.danforthcenter.org/yu/pdf/e-flower-2006.pdf</a>

As example the following cross has been chosen: Rosea x F. fulgens grandiflora. I raised 25 cultivars from this cross and all these seedlings were examined by HPLC. For results see tables below and/or page pigmentanalyse: <a href="http://members.home.nl/henkwaldenmaier/pigmentanalyse.htm">http://members.home.nl/henkwaldenmaier/pigmentanalyse.htm</a> .

## Found pigments in petals of Rosea x F. fulgens grandiflora:

Rel.	Seedling	P <i>G</i> 35	<i>C</i> Y35	PN35	DP35	PT35	MV35	Σ	PG3	DP3	PT3	MV	Σ	petal color	Total
No.								35				3	3		pigment
1	B89-958	31		27			9	67				9	9	orange	297
2	B89-980	55		13	3			71				13	13	orange	501
	= WALZ														
	Lucifer														
3	B89-945	56		10				66	4			11	15	orange	279
4	B89-971	50		27				77	4			6	10	orange	366
5	B89-948	10		54				64			6		6	lilac/red	418
6	B89-959	3		26			38	67	3				3	lilac/pink	963
7	B89-942	48		9				57				17	17	orange	301
	=WALZ														
8	Wolkbreuk B89-967	14		50			17	81					0	lilac/red/orange	595
9	B89-946	4	1	35			42	82	1				1	red/purple	1361
10	B89-974	6	1	36			37	80	2				2	dark red	1392
11	B89-955	63	1	9			12	84	3			8	11	dark red dark orange	663
12	B89-932	5	1	44			29	79	1		1	0	2	lilac/red	1001
13	B89-972	56	1	18			9	83	2		1	7	9	red/orange	969
14	B89-968	48		9			8	65				9	9	lilac/red/orange	750
15	B89-973	3	1	27	2		38	71	2			9	2	purple/red	1719
16	B89-944	5	2	55			17	79					0	red	1121
17	B89-934	11	2	32	1		42	88	2				2	dark red	2001
18	B89-983	52	3	20	1		42	75	6			5	11	lilac/red/orange	491
19	B89-947	49	3	18			9	76	8			13	13	lilac/red/orange	300
20	B89-952	49		17			10	72	2	3		9	14	lilac/red/orange	651
21	B89-979	45	4	33	2	10	38	87	1	3		9	14	lilac/red	1122
22	B89-981	45	2	10	2	10	8	67	5	2		9	16	lilac/red/orange	452
23	B89-961	5		27			0	32	5		42	9	42	dark red	320
24	B89-965	14		39			38	91			42		0	red	591
		14		46			38	-			22				
25	B89-935	14		46				60			23		23	salmon/pink	408

## Found 'other' pigments in petals of Rosea $\times$ F. fulgens grandiflora:

Seedling	X04	X07	X08	X09	X10	X11	X12	X13	Σ
									×
B89-958							13	11	24
B89-980				7				9	16
= WALZ Lucifer									
B89-945					7			11	18
B89-971								12	12
B89-948	6		5	5				15	30
B89-959	8		5	3				14	30
B89-942				5				21	27
= WALZ Wolkbreuk									
B89-967	2		3	2				11	19
B89-946	3	2	2					10	17
B89-974	5		2	5				6	18
B89-955								6	6
B89-932	3	1	4	2				7	17
B89-972						1		7	8
B89-968	2		4	6		2		5	19
B89-973	7		4	5				12	28
B89-944	2	2	4	5				8	21
B89-934	3			1				5	10
B89-983			3	3				8	13
B89-947								11	11
B89-952				3			4	5	13
B89-979	2		2					9	13
B89-981			5	10				3	17
B89-975				5	8			14	26
B89-965								9	9
B89-935								17	17

The best approach would be to score the seedlings on the basis of found anthocyanins. However, since chemical analysis of pigments present in cultivars is difficult to perform, and consequently not practical, classification for F1GenCalc was done based on the phenotypic outcome of the cultivars.

This resulted in the following table (table A):

Color phenotype	Rel. seedling Nos.	Total number	%	Pigment
				class
orange	1-2-3-4-7-11	6	24	PG = 28%
red-orange	13	1	4	
salmonpink	25	1	4	
lilac-red-orange	8-14-18-19-20-22	6	24	<i>C</i> Y = 44%
lilac red/pink	5-6-12-21	4	16	
red	10-16-17-23-24	5	20	DP = 28%
redpurple	9-15	2	8	

## Genotypic identification:

There are two loci: F3H (hydrolysis at R1) and F35H (hydrolysis on [part of R1 and] R2). For F1GenCalc these loci are coded A and B respectively.

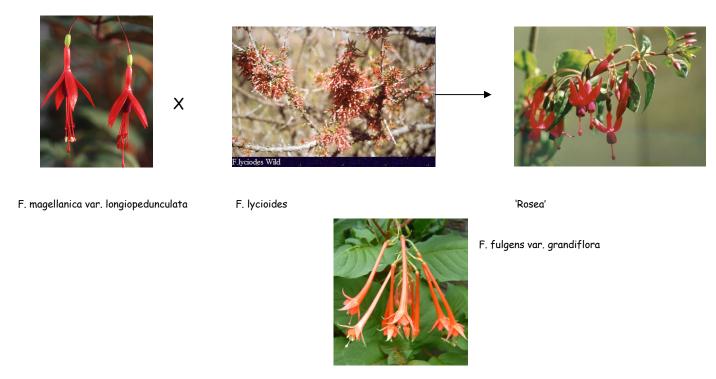
Locus	Allel	hydrolysis		
F3H	H A 100% OH on R1 a1-a9 Partly OH on R1			
	α	No OH on R1		
F35H	В	100% OH on R2		
	b1-b9	Partly OH on R2		
	b	No OH on R2		

Rosea is a cross between F. magellanica and F. lycioides.

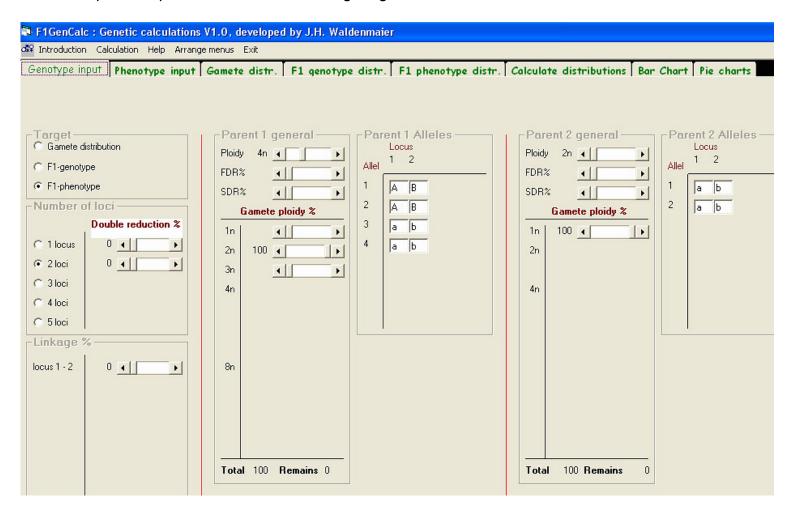
F. magellanica var. longipedunculata is a tetraploid species with 50% MV3G5G pigment (and 50% others) in the purple petals. Genotype AAAABBBB.

F. lycioides is a tetraploid species with orangepink flowers. No pigment analysis was performed. This species has its original habitat in the desert (exceptional for fuchsia species). This might be the reason why the orange color is slightly moved to the pink direction (pH?); Genotype aaaabbbb. 'Rosea': genotype 100% AAaaBBbb (AAAABBBB x aaaabbbb), actual pigments found: 25% PN3G5G, 50% MV3G5G, 25% others.

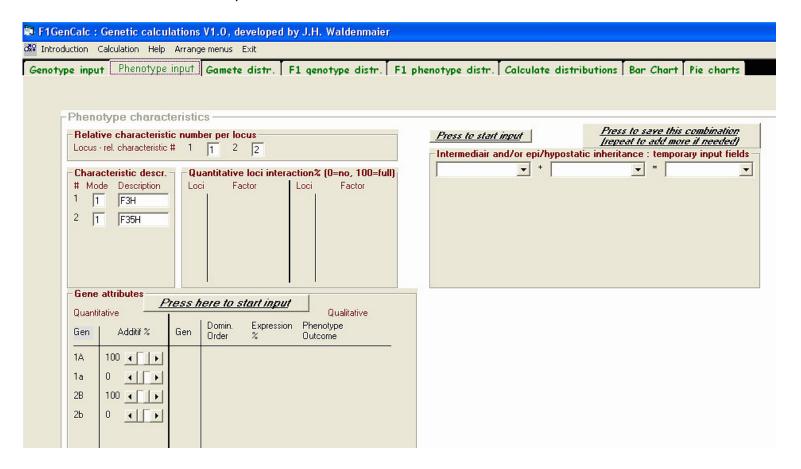
F. fulgens grandiflora is a diploid species with 100% PG3G pigment in the orange petals. Genotype aabb.



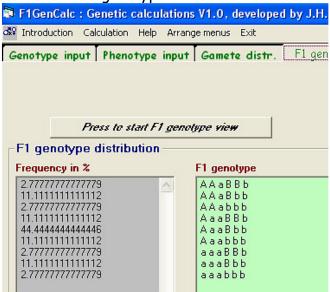
## F1GenCalc parent input screen ('Rosea' x F. fulgens grandiflora):



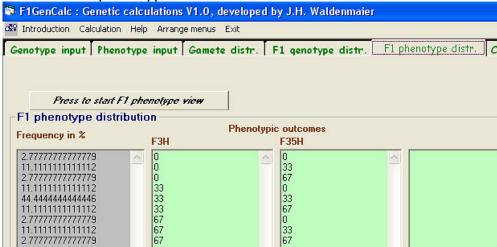
## F1GenCalc inheritance mode input screen:



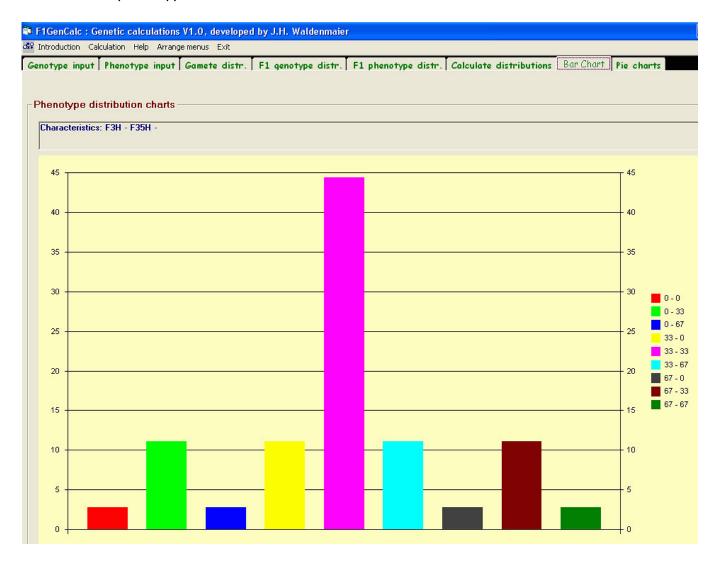
### F1GenCalc F1 genotype screen:



## F1GenCalc F1 phenotype screen:



## F1GenCalc F1 phenotype barchart screen:



Conversion of F1GenCalc F1 geno/phenotypes to real phenotypes and check against actual phenotypes:

F1GenCalc					Actual
Genotype	Phenotype	% Color		Σ%	(see table A)
	F3H-F35H		(estimation)		
aaabbb	0-0	2.8	orange		
aaaBbb	0-33	11.1	orange	27.8	28
aaaBBb	0-67	2.8	orange		
Aaabbb	33-0	11.1	orange/pink		
AaaBbb	33-33	44.4	lilac-red-orange	44.4	44
AaaBBb	33-67	11.1	lilacred		
AAabbb	67-0	2.8	red	27.8	28
<i>AA</i> aBbb	67-33	11.1	redpurple		
AAaBBb	67-67	2.8	purple		



## Fuchsia flower type (single, semidouble, double)

Fuchsia's have from origin 4 petals in the flower. Due to mutation this number can change from 0 to 8 or more.

Number of petals	Flower type	
0	Apetalous	
1-3	Single	
4	Single	
5-7	Semidouble	
8	Double	
>8 (mostly in sets of 4)	Double	

An example of apetalous fuchsia's are the species from the section hemsleyella



species F. juntasensis (fuchsia section Hemsleyella)

Double-flower forms often arise when some or all of the stamens in a flower are replaced by petals. These types of mutations, where one organ in a developing organism is replaced with another, are known as homeotic mutations. They are usually recessive and cause infertility (for example double tuberous begonias). I sometimes have observed this phenomenon in my fuchsia-crossings (mostly 4 of the eight stamens were replaced by small petal-like leaves). Also petaloids (connected to the sepals) are sometimes present.

These mutations are NOT the mutations I want to address to in this chapter.

The mutation resulting in double flowers I will dealt with here is a duplication/deletion point mutation of the gen responsible for the presence of the normal number of petals. All other flower parts (such as stamens) are in principle unaffected.

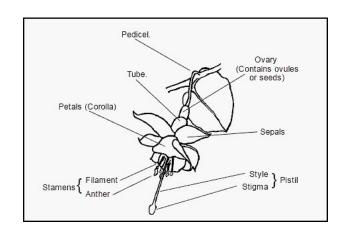
This shows the consequences of errors in crossing-over after chromosome pairing during meiosis.



Let us assume this gen (red part in above scheme: for the presence of the normal number of petals) is coded p4. The duplicated version is coded as p8, whereas the deleted version is coded as p0. The mode of inheritance is quantitative, that means that the different alleles in the genotype might be summed up to get the phenotype.

It should be taken in mind that the characteristic flowertype can be influenced by growing conditions. Double flowers can show semi-double under bad conditions (eg late in the flowering season), nearly semi-doubles can show as semi-double and nearly doubles as doubles under very good conditions.

Fuchsia flower scheme









DOUBLE

Examples of fuchsia flower (note the presence of all stamens and pistil in the double flowers)

#### Some history

The coming into existance of double flowering fuchsia's and which genes were involved in that process was published by Victor Reiter Jr. (Journal of the California Horticultural Society, volume V No. 4, 149-150, October 1944). He wrote the following:

The doubling mutation was probably a gradual improvement arising from the very mediocre beginnings. The mutation for doubleness usually accelerates as breeding continues and a tendency is all that seems needed at the outset. Porcher sets the date of the first introductions at 1850 and suspects that the doubling tendency arose out of Corallino (radicans x exoniensis). The first two double varieties, Duplex and Multiplex (Story), according to Porcher were double but the petals were hidden and the mediocrity of their flowers resulted in their neglect. So intense was the breeding work on these doubles that by 1865 Bull had introduced Gypsy Queen. Less than fifteen years and probably fewer than seven generations of breeding had passed since Duplex and Multiplex.

Nb. For the parentage of Corallino nowadays we should say
Radicans = F. regia (octaploid)
Exoniensis = F. magellanica globosa (tetraploid) x F. cordiofolia (diploid)

If the mutation from p4 into p8 occurred in the polyploid Corallino (heptaploid?), probably one of the alleles from Corallino had the p8 allel, and all other alleles the original p4 allel, reason why Corallino was single in phenotype. Inbreeding on this p8 allel resulted in cultivars with a high % of p8-alleles with double flowers.

Actual crosses have led to the theory, that when no p4 and no p8 alleles are present (only p0 present), the cultivar is apetalous, (very) low percentages of p4-alleles give flowers with 1-3 petals, and cultivars with a high % of p8-alleles give flowers that are (semi)double.

Without knowledge of the genotypes, the following actual fuchsia crosses give insight in the underlying mechanism:

phenotype parents	seedlings				
	% single	% semidouble	% double	number	
single x single	69.5	4.6	25.8	296	
single x semidouble	72.2	16.6	11.1	38	
single x double	64.8	9.7	25.4	260	
semidouble x semidouble	67.5	13.5	18.9	37	
semidouble x double	12.9	14.1	72.9	85	
double x double	13.3	7.0	79.6	157	
Total	53	9	38	873	

#### Some conclusions from this table:

- single x single gives a quarter doubles (some singles are heterozygotes, one dominant-recessive gen involved)
- double x double gives single cultivars (some doubles are heterozygotes, dominance of single is only partly dominant)
- single x single, single x double, single x semidouble and semidouble x semidouble give about the same phenotype frequencies (polyploid additive quantitative inheritance)

## <u>Proposed model for translation of genotype into phenotype:</u>

allel	Contribution to	
	doubled flowers	
pO	0	
p4	50	
p8	100	
Possibly p12 etc. (in case of	150 etc.	
repeated duplication mutations)		

Table B

Sum of contribution in genotype to doubled flowers	phenotype
0	apetalous
>=0 and <=10	1-3 petals
>10 and <80	single
>=80 and <=82.5	semidouble
<b>&gt;</b> 82.5	double
>100	very double

The existence of one locus for as well the duplication as the deletion results also when analyzing the following primary crosses:



 $\frac{\text{Tetraploid F. splendens (single)} \times \text{diploid F. perscandens (apetalous)} :}{100\% \text{ single F1 cultivars}}$ 

Single primary cross (triploid), doubled with Colchicin treatment to hexaploid, with 4 petals and full set of stamens and pistil

## <u>Tetraploid single F. magdalenae x tetraploid (after Colchine treatment) apetalous F. excorticata:</u> 100% single F1 cultivars (total of 40 seedlings)



Tetraploid single F. magdalenae

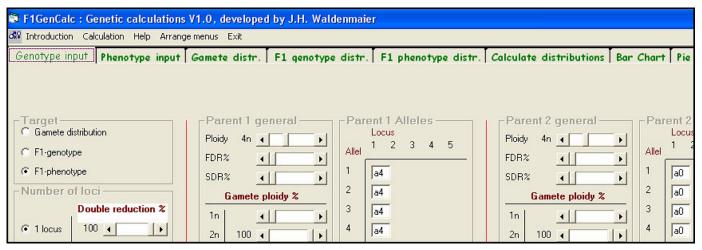


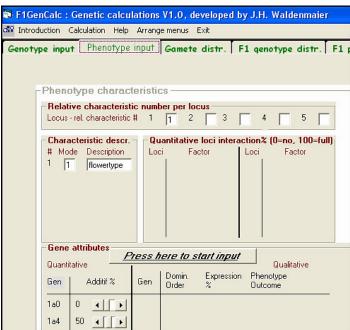
Tetraploid apetalous F. excorticata

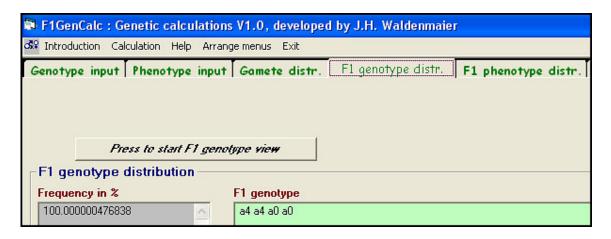


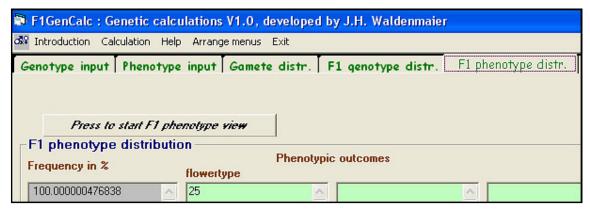
Tetraploid single F1-cross

#### F1GenCalc screens:









See table B: sum of contributions to doubled flower of 25 has a single flower phenotype. So the prediction of 100 % single equals the actual % of 100%.

## Cross of single F. magdalenae (see above) X double cultivar Vanity Fair



F. magdalenae

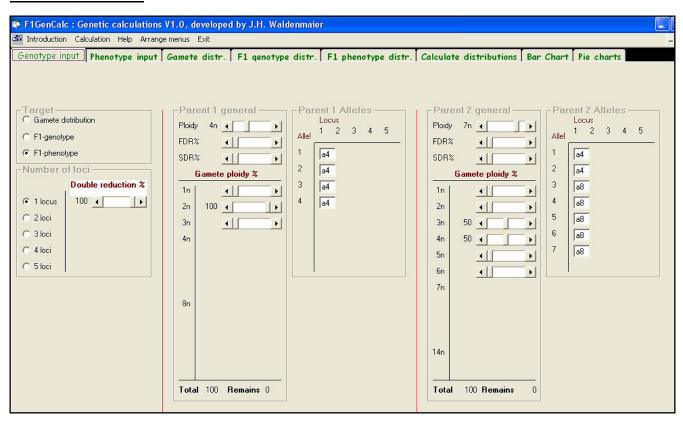
Vanity Fair

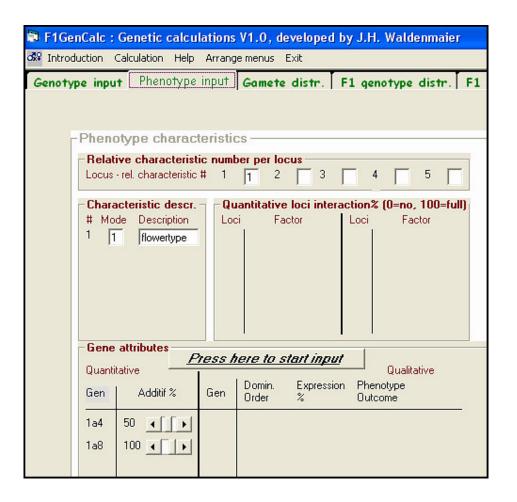
<u>Fuchsia</u>	<u>Genotype</u>	<u>flowertype</u>
F. magdalenae	P4 p4 p4 p4	single
Vanity Fair	P4 p4 p8 p8 p8 p8 p8 (estimate)	double

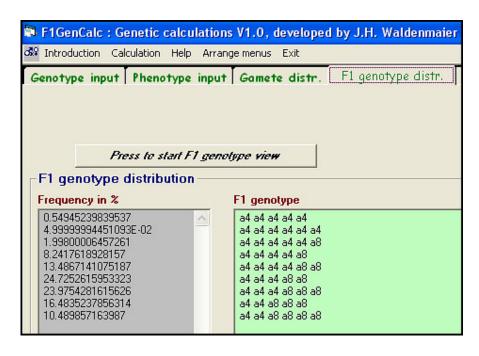
#### Table C:

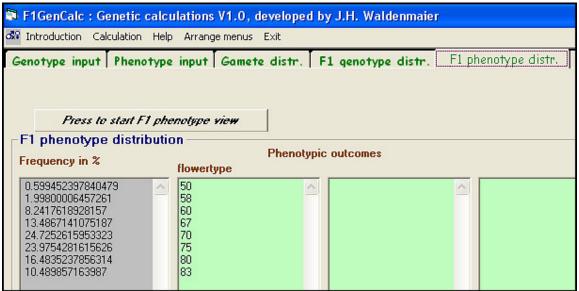
Actual phenotype	%	N seedlings		
single	70	23		
semidouble	18	6		
double	12	4		

## F1GenCalc screens:









## $\underline{\textit{F1GenCalc} \ predicted \ phenotypes \ translated \ into \ flower type \ phenotypes:}$

F1GenCalc phenotype				Actual phenotype	
F1GenCalc type	Frequency	Summed	Flower	Frequency	Flowertype
	In %	Frequency in %	phenotype	In %	
50	0.6				
58	2.0				
60	8.2				
67	13.5	73.0	single	70	single
70	24.7				
75	24.0				
80	16.5	16.5	semidouble	18	semidouble
83	10.5	10.5	double	12	double

Taken into consideration the relative small number of seedlings (33) this prediction can be qualified as 'good'.