Clivia Mutations and Colour Variation
By Wessel Lötter

1. Definitions and Terminology

Flavonoids: Pigments dissolved in the cell sap, such as anthocyanidins, which are red or blue, betaxanthins, which are yellow and anthoxanthins which are yellow or cream. Unrelated compounds are betacyanins of which betanidin found in beet is an example.

Carotenoids: Pigments in plastids within the plant cell, namely carotenes, which are orange and xanthophylls, which are yellow.

True yellow: For the purpose of this article, true yellow shall be a clivia, which displays a complete lack of anthocyanin in its flowers, berries and seedlings.

Par-yellow: This plant is still able to produce very little anthocyanin, which sometimes shows up as spots or streaks on the flowers and / or berries.

The seedlings however are unpigmented. It has only recently been identified as a second mutation. The term "par" is not my creation but an already established term in some bird mutations e.g. blue and par-blue, which are not equal in colour but equal in its inheritance (recessive over the normal green colour). Further in birds where the colour is equal and the inheritance differ, the mode of inheritance is used to differentiate between the two e.g. sex-linked yellow and recessive yellow. The par-yellow clivias are definitely not freaks or rogues but merely a mutation of another gene in the anthocyanin pathway **(Figure 1)**. Natal Yellow A and B are examples.

True breeding: A phenotype can only be true breeding if it is homozygous. This means that a true yellow of the genotype aaJJ or a par-yellow of the genotype AAjj will be true breeding if selfed or crossed with its own genotype. A true yellow of the genotype aaJj is heterozygous (carrying factor for par-yellow), and if selfed will produce three different genotypes. Genetically it will not be regarded as true breeding even if its phenotype remains unaltered. The three different genotypes are aaJJ, aaJj and aajj. The latter can be crossed with true yellow or par-yellow producing 100% yellow progeny, true yellow or par-yellow, as the case may be of the genotypes aaJj and Aajj respectively. If these two genotypes are crossed 25% orange will be produced as will be demonstrated in the concluding section of this article. The term true breeding can only be used if both parents are known to be of the same homozygous genotype.

2. Combined Mutations

At the meeting on the 14th February 1998, I demonstrated three different methods to indicate how I arrived at my prediction as set out in item 10 of my article (Newsletter vol. 7, Jan. 1998, p.13). I was asked to do an article on this for the newsletter, but I was reluctant to do so, as I had no literature to substantiate my views. I was fortunate enough to receive an article by R.J. Griesbach of the United States Department of Agricultural Research Service, Beltsville, Maryland, 20705, under the heading "Genetics and Biochemistry of Anthocyanin Albinism in *Paphiopedilums* and I quote:

"Most plants are diploid and have two copies of every gene. For discussion purposes, *Paph. faireanum* fma. *alba*'s genotype could be designated AABBCCDDEEFFGGHHjj.

The two copies of the "j" anthocyanin gene are non-functional and anthocyanin is not produced. On the other hand one could designate *Paph. bellatum* fma. *alba*'s genotype as aaBBCCDDEEFFGGHHIIJJ. In this case, the two copies of the "a" anthocyanin gene are non- functional. The hybrid Paph. x Iona, would have one gene from each parent and would be designated AaBBCCDDEEFFGGHHIIJj. In Iona, there would be at least one functional copy of each anthocyanin gene, which would result in red flowers. Two copies of each gene are not needed for

anthocyanin production. When Paph. x Iona is selfed, one would find that 7/16 or 44% of the progeny are alba (Table 1)

Table 1. Genetics of diploid Paph. x Iona (AaJj) = *Paph. faireanum alba* (AAjj) x *bellatum alba* (aaJJ).

Parents F1: AaJj x AaJj

Sex cells:

25% AJ 25% AJ

25% Aj 25% Aj

25% aJ 25% aJ

25% aj 25% aj

Progeny F2:	**AJ**	**Aj**	**aJ**	**aj**
AJ	**AAJJ**	**AAJj**	**AaJJ**	**AaJj**
Aj	**AAJj**	**AAjj**	**AaJj**	**Aajj**
aJ	**AaJJ**	**AaJj**	**aaJJ**	**aaJj**
aj	**AaJj**	**Aajj**	**aaJj**	**aajj**

Alba genotypes = AAjj, Aajj, aaJJ, aaJj, aajj.

Figure 1: Anthocyanin biosynthetic pathway showing the action of ten genes (G. Stotz, et al.,1985. *Theor. Appl. Genet.*70:300).

P – coumaric acid –A- Chalcone -B- Naringenin -C -Dihydrokaempferol / Eriodictyol

-D- Dihydroquercetin- E,F,G,H,I,J- Acylated Anthocyanin.

Table 1 by Griesbach as set out above was one of the methods demonstrated by me at the meeting on the 14th February 1998, with the only difference being the symbols I used. Apart from the 7/16 or 44% (to the nearest) albino progeny, Griesbach did not give a full analysis of all the genotypes and their percentages. For convenience I shall use his symbols for our yellow clivias i.e.

P-generation: aaJJ for true yellow. x AAjj for par-yellow.

F1-generation: AaJj F1-orange

These F1-orange parents will give rise to the following genotypes in the F2-generation.

F2-generation:

1. AAJJ 1/16 6.25% Orange

2. AAJj 2/16 12.5% Orange split par-yellow

3. AaJJ 2/16 12.5% Orange split true yellow

4. AaJj 4/16 25% Orange split true and par-yellow

5. AAjj 1/16 6.25% Par-yellow

6. Aajj 2/16 12.5% Par-yellow split true yellow

7. aaJJ 1/16 6.25% True yellow

8. aaJj 2/16 12.5% True yellow split par-yellow

9. aajj 1/16 6.25% True yellow and par-yellow combined.

Genotypes 1-4 are orange and represent a total of 56.25%.

Genotypes 5-9 are yellow and represent 43.75% in total.

Phenotypic ratio of 9:7

The genotype in item 9 above is true yellow as well as par-yellow, but its phenotype is true yellow only. The reason for this is that the anthocyanin pathway is totally blocked by the genes responsible for true yellow and thus the phenotypic expression of the genes responsible for par-yellow is suppressed. This is called epistasis or masking. There are several forms of epistasis. They have one thing in common and that is that they change the normal expected phenotypic ratio of breeding results considerably. The British geneticist Bateson discovered this particular form involving the inter-action of two different recessive gene pairs, early in the 20th century. He crossed two true breeding sweet peas with white flowers. To his surprise this resulted in purple flowers only. This F1-generation was allowed to self and out of a total of 651 F2-plants, 382 produced purple flowers and 269 white flowers, a ratio of 9:7. The following table was used to demonstrate the interaction of the genes and the 9:7 ratio.

P-generation: aaBB (White) x AAbb (White)

or alternatively: AABB (Purple) x aabb (White)

Gametes: **(sex cells)** aB and Ab

F1-generation: AaBb (Purple)

F2-generation: AaBb x AaBb

Gametes	**AB**	**Ab**	**aB**	**ab**
AB	**AABB**	**AABb**	**AaBB**	**AaBb**
Ab	**AABb**	**Aabb**	**AaBb**	**Aabb**
aB	**AaBB**	**AaBb**	**aaBB**	**aaBb**
ab	**AaBb**	**Aabb**	**aaBb**	**aabb**

F2-generation: Phenotypic ratio of 9 purple and 7 white.

This deviates from the normal expected phenotypic ratio of 3:1 in the F2-generation when a single recessive gene pair is involved.

Example (as for clivias)

AA (orange) x aa (yellow) = 100% Aa F1-orange.

Aa x Aa = 25% AA (orange), 50% Aa (orange) and 25% aa (yellow).

F2-generation = 75% orange and 25% yellow a ratio of 3:1

3. Aberrant Yellow Forms

A Clivia is variable in many respects and colour is no exception. For example it may be able to produce anthocyanin in the normal way, but the intensity of the colour as well as the extension thereof, wherever it may occur in the plant, may vary considerably. In Miriam Meltzer's vast collection of some 50 000 orange clivias, mostly grown from seed, there are two or three with orange flowers and yellow berries. It is not surprising that in this vast collection three yellows arose, but with a difference. The one has orange midribs on the outside of its petals, the other one is the picotee as illustrated in the Clivia Review 1998 and the third one is a clear yellow with orange berries.

Further more Nick Primich produced a yellow from a normal orange plant. He selfed this yellow and I know he does this meticulously, but the seedlings were all pigmented. Hopefully we will now be able to see whether some of these pigmented seedlings will produce yellow flowers. Miriam Meltzer's seedlings from her yellows are also all pigmented. I myself used the pollen of the picotee on a yellow with faint red tips to its petals, but the progeny are all pigmented. I am now more than ever before convinced that a plant with normal functioning anthocyanin can produce yellow flowers in as much as an orange flowering plant can produce yellow berries.

4. Dilute

This is another phenotype, which is closely related to our yellow mutations. In a dilute the intensity of the pigmentation is so diluted that very pale flowers result. Chubb's peach is a good example of this.

By analyzing the breeding behavior of a plant, its genotype can be deduced. The following breeding results were obtained from Chubb's peach:

(a) Natal Yellow B x Chubb's peach = 100% orange flowering plants.

(b) True yellow x Chubb's peach = 100% unpigmented seedlings.

This implies that the defect, which is responsible for Chubb's peach, acts on the same gene as that which is responsible for true yellow. Experience with other genera of plants has proved that a normal plant is dominant over both dilute and albino, but that dilute in turn is dominant over albino. As Chubb's peach originated in an orange flowering population where no yellows occur, the genotype **A'A'** could be designated to it. **A'A** would be orange flowering as orange is dominant over dilute. The genotype of the progeny in (a) above would therefore be **A'AJj (Orange)**. The genotype of the progeny in (b) would be **A'a**, and therefore all dilutes, as **A' (dilute)** is dominant over **a (true yellow)**. If the dilute of the genotype **A'a**, is crossed with a true yellow **(aa)**, 50% dilute and 50% true yellow would be produced.

<u>Example:</u>

	a	**a**
A'	**A'a**	**A'a**
a	**aa**	**aa**

50% dilute and 50% yellow

The origin of Naude's peach is unknown and so is its genotype. Preliminary experiments indicate that it could be of the same genotype as Chubb's peach, but further research is necessary to confirm this. Few of the ten genes in the anthocyanin pathway give rise to viable mutants for reasons as set out in the next section dealing with white. We already have two of the ten genes responsible for clivia mutations i.e. one for true yellow and Chubb's peach and one for par-yellow. The possibility of a third gene being involved is therefore unlikely. The allelic relationship (being different forms of the same gene) of Naude's peach with par-yellow has been ruled out. The following breeding results confirm this and suggest a relationship with true yellow and Chubb's peach:

(a) Natal yellow x Naude's peach: 100% pigmented seedling.

(b) Natal Yellow x Chubb's peach (F1-orange) x Naude's peach: 5 seedlings – 3 pigmented and 2 unpigmented.

(c) True yellow x Natal yellow B (F1-orange) x Naude's peach: 18 seedlings – 12 pigmented and 6 unpigmented. This deviation from the expected 50% may be due to the fact that one third of the flowers of the seed plant were already open when pollination with Naude's peach commenced.

Meg Hart unintentionally and without knowing which the parents were, bred three dilutes. Either she must have an orange flowering plant of the genotype **A'A**, which selfed or a peach which is so diluted that it appears yellow.

Dilute should not be confused with pastels, which are very pale orange forms. Pastels can be crossed with any colour within the orange spectrum producing a variety of shades. It is not yet known whether Meyer's Peach is a dilute or a very pale pastel. If pollen can be made available, the necessary experiments will be done to determine its genotype.

5. The Mythical White

Griesbach defines an albino as follows: "An albino will by definition display a lack of anthocyanin pigmentation, but will contain chlorophyll and carotenoid pigments". Our yellow clivias are therefore already albinos. The yellow colour of the flowers is mainly due to carotenoids, which are also present in the leaves. Without this the plant cannot survive. It not only protects the chlorophyll, but is also involved in the absorption of certain rays of the sun. Further more, the vast majority of albinotic mutations are blocked at the same step in the anthocyanin pathway. The reason for this is that the ten genes in the anthocyanin pathway are also involved in other flavanoid pathways, which are essential for growth and protection from the harmful effects of ultra-violet irradiation. Admittedly yellow pigments are not essential in the flower and it may be possible to produce a white by selective breeding.

The clivia with the white and green center as depicted on page 6 of the Clivia Review 1998, preferably as a mother plant, would be an excellent choice.

By crossing this plant with yellow you may get whites or nearly so, with green in the center in the F2-generation. If I had a choice for a yellow as pollen plant, it would be one which already has green in the center, like that on page 8 of the Clivia Review 1998.

The reason why this breeding should not be done the other way round (yellow as mother plant) is because yellow is said to be maternal and may be a dominating factor if you have white in mind.

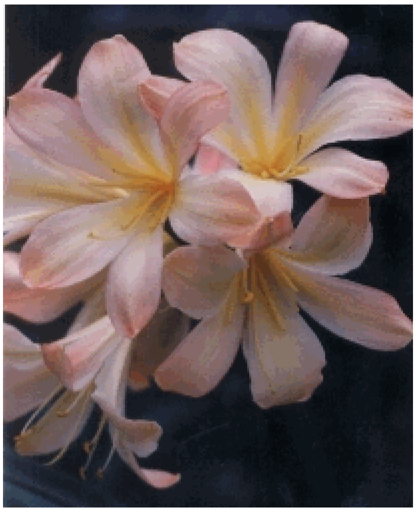
6. Colour

Colour in the clivia is the combined effect of the presence of anthocyanin in the epidermis and xanthophylls in the mesophyll (underlying tissue). Removing the epidermis of an orange flower can prove this. The epidermis will be red and the exposed mesophyll will be yellow. If this red epidermis is placed on any yellow surface, the colour will change to orange. The different shades from pastel to red are the result of the ratio of cells in the mesophyll, containing leucoplasts that are colourless, chromoplasts that contain yellow xanthophylls and the concentration of anthocyanin (red pigment) in the epidermis.

Example:

A flower with few chromoplasts and a high concentration of anthocyanin will be red.

A flower with few chromoplasts and a low concentration of anthocyanin will be pink.



A flower with leucoplasts only and no anthocyanin will be white. (No photo available)

A flower with chromoplasts in abundance and a low concentration of anthocyanin is salmon.

The epidermis of a yellow clivia flower contains no anthocyanin and is transparent. The yellow colour is displayed by carotenoids in the mesophyll underneath.

The colour of the berries is influenced by an additional factor namely: chlorophyll, which is retained in the mesocarp (pulp) of some plants.

<u>Example:</u>

Orange – red berries have yellow pulp and a high concentration of anthocyanin in its epidermis.

Yellow berries have yellow pulp and a transparent epidermis.

Pale green berries have green pulp and a transparent epidermis.

Purple berries have green pulp and a red epidermis. If the epidermis of this berry is removed and thoroughly washed it will be red. Place it on a green surface, press it well down and it will appear purple again.

7. <u>Conclusion</u>

This study has proved that there is not a pair of genes for red and a pair of genes for yellow as previously accepted. Yellow is due to a gene, which is responsible for one of the enzymes necessary for the production of anthocyanin, being non-functional. In the case of dilute the gene is defective. Furthermore the theory of the hidden red gene can now also be explained. We may already have yellows of five different genotypes namely: **aaJJ, aaJj, aajj, AAjj and Aajj.**

Not all of these would produce 100% yellow progeny. We already know that true yellow **(aaJJ)** x par-yellow **(AAjj)** produce 100% orange, but **aaJJ x Aajj** would produce 50% orange and 50% yellow, whereas **Aajj x aaJj** would produce 25% Orange and 75% yellow progeny.

<u>Example 1:</u>

P-generation: aaJJ x Aajj

Gametes: aJ aJ Aj aj

Progeny:

	Aj	**aj**
aJ	**AaJj**	**aaJj**
aJ	**AaJj**	**aaJj**

50% orange and 50% yellow

<u>Example 2:</u>

P-generation: Aajj x aaJj

Gametes: Aj aj aJ aj

Progeny:

	aJ	**aj**

Aj	**AaJj**	**Aajj**
aj	**aaJj**	**aajj**

Yellow genotypes Aajj, aaJj and aajj = 75%. Orange genotype AaJj = 25%

8. Bibliography

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