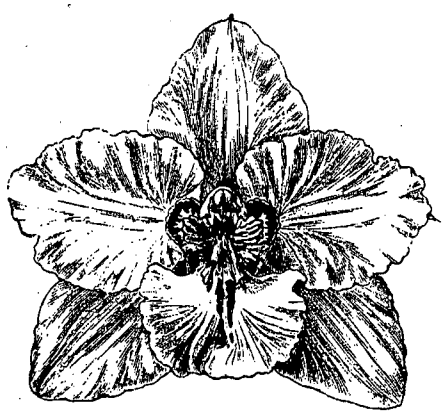
Odontoglossum Alliance Newsletter

Volume 4 February 2002

Program and Annual Meeting Odontoglossum Alliance



The annual meting of the Odontoglossum Alliance will be held on the afternoon of 12 April 2002. This meeting will be held in conjunction with the AOS Trustees meeting and the Illinois Orchid Show 10-14 April 2002.
There is a lecture program of four speakers and an evening dinner at a Froggy's, a fine French restaurant. The meeting will be held in the **Renaissance Chicago North Shore Hotel** at 933 Skokie Blvd, Northbrook, IL 60062, Phone number 847-498-6500. There is a limited block of rooms with a rate of $99.00 per night. After that block is filled the next rate is $109.00/night. This hotel is in the **Marriott Hotel Company**. **Please note that the last newsletter listed this as Sheraton. It is NOT Sheraton.**
Transportation from O'Hare Airport can be by Continental Airport Express @ $20.00 per person. There is a courtesy phone or call 800-657-7871. For $24.00, American taxi has a courtesy phone or 800-244-1177. Enterprise rent-a-car is located at the hotel. Should you come into Midway Airport it is about 30 miles from the hotel while O'Hare is about 15 miles from the hotel.

Program

1:00 PM 12 April 2002
Location: A Meeting Room in the Hotel

Session Chairperson: Sue Golan

1:15PM –2:00 PM Larry Sanford, Cincinnati, Ohio;

Title: "Leonore and Milton Are Both Right!"
Some observations and measurements about growing cool Odonts and their warmer growing intergenerics in the Ohio Valley.

Larry Sanford.

Married to Mary Patience Rood and celebrated our Fifty Fourth anniversary last March. Patience is truly a wife for all seasons as well as the inspiration for awarded plant clonal names.

Current cool greenhouse is about 8 feet by 15 feet: small enough to refrigerate in the summer and to light with HP sodium for morning 'kick start' and dreary winter days.

Multiple American Orchid Society Plant Quality Awards in the Odont Alliance; Accredited Judge;

amateur hybridizer with about 15 crosses in progress.

Born and raised in Tucson, Arizona; WW II Navy, BSCE University of Arizona, 1950, Ph.D. Cornell University, 1955: joined Proctor and Gamble 1955; retired in 1990; Inducted into the Paper Industry Hall of Fame 1997 for work in P&G's Paper Division.

2:00-2:45 PM Milton Carpenter, Everglades Orchids, Belle Glade, Florida,

Title: " Creating Odontoglossum Alliance Hybrids for Tropical and Sub-Tropical Climates"

This program will explore the speaker's 40-year quest in the creation of Odontoglossum alliance hybrids, which will perform well in warmer climatic areas of the world while retaining a substantial percentage of the beauty seen in modern "cool-growing" members of this alliance.

With well over 10,000 hybrid attempts as a basis for comparison, this speakers experiences along "the road less traveled" will be documented by both word and image.

Milton O. Carpenter – Brief Orchid Biography

A native of the Florida Everglades, Milton has been growing orchids for 40 years and is the owner of Everglades Orchids, Inc. in Belle Glade, Florida.

He is Past President and Life member of the Orchid Society of the Palm Beaches. He is also Immediate Past President, Trustee, Life member, and accredited judge of the American Orchid Society.

Milton is a world renowned speaker, author, hybridizer, grower, photographer and explorer, having made may trips to different countries on the world to study and photograph orchids in their habitat.

His quest in hybridizing has been within the Oncidiinae and Cymbidiinae, which will thrive, in warm as well as cool conditions.

2:45 – 3:15 Break

3:15 – 4:00 Professor Norris Williams, University of Florida, Gainesville, Florida

Title: "Molecular Systematics (DNA) of the Odontoglossum Alliance

What is an *Oncidium* or an *Odontoglossum* has been a problem in orchid taxonomy for over 100 years. Species have been described in one or the other genera and moved back and forth depending on the whims of various taxonomists. Modern techniques using molecular genetics offer an objective method of resolving the conflict and defining these groups of species and their relatives. I have increased sampling in the Oncidiinae to 522 species representing 84 generic concepts for one nuclear region (ITS) and 138 species for additional plastid regions (*matK* and *trnL-F*). The ITS results confirm the non-monophyletic nature of *Oncidium* and suggest *Cochlioda, Collarestuartense, Mexicoa, Miltonioides, Odontoglossum, Sigmatostalix, Solenidiopsis,* and *Symphyglossum* should be merged into *Oncidium*. The phylogenetic relationships of the subtribe Oncidiinae will be discussed, with particular emphasis on the *Oncidium/Odontoglossum* problem.

Norris H. Williams

He was born and grew up in northern Alabama, received his BS and MS degrees in biology from the University of Alabama, and attended Washington University in St. Louis for one year. While at Washington University, he took a course in Tropical Ecology and went to Panama for a three-week field trip, where he was

introduced to Bob Dressler and the fascinating field of orchid pollination biology. He left Washington University and went to the University of Miami to study orchids with Cal Dodson in 1966. He spent 1968-69 living in Panama and working with Dressler at the Smithsonian Tropical Research Institute, and received his PhD in 1971. After that he spent one year at the Smithsonian Institution working on anatomy of orchids, then a year at Fairchild Tropical Garden continuing work on orchid anatomy and systematics. He went to Florida State University in 1973 as an Assistant Professor, was promoted to Associate Professor in 1978, and in 1981 moved to the University of Florida as Keeper of the Herbarium. He is currently Keeper of the Herbarium, Curator of Vascular Plants, and Joint Professor of Botany at the University of Florida.

He has traveled extensively in Central and South America studying orchids for the past 37 years. He spent 30 years mainly working on the chemistry of floral fragrances, pollination biology of orchids, and systematics and evolution of orchids. Six years ago he began working on molecular systematics of orchids, with initial funding for his work on the Oncidiinae from the American Orchid Society and later the National Science Foundation. His current research is on the molecular systematics of a variety of groups of orchids, especially those of the American tropics. In collaboration with several other scientists, they are working to establish a stable system of classification of the Orchidaceae based on objective criteria, rather than appeals to authority.

4:00 –4:45 PM Stig Dalström, Marie Selby Gardens, Sarasota, Florida

Title: "When One and One Becomes Three, At Least"

A discussion, with slide presentation, about nomenclatural confusion in Oncidiinae, with examples of how miscommunication and misunderstanding lead to synonymy and other unfortunate errors in orchid classification. Also a discussion about distribution and speciation patterns in Odontoglossum and Cyrtochilum.

Stig Dalström

"Alien with extraordinary ability" (US Immigration)

Stig Dalström is a self-taught artist, illustrator and orchid taxonomist. Born and raised in Sweden, he now resides in Sarasota, Florida, US. He works for Marie Selby Botanical Gardens, and as a freelancer.

His scientific work covers much of the Andean species of Oncidiinae, particularly genera such as Cochlioda, Cyrtochilum, Odontoglossum and Oncidium.

His fine art can be seen in public and private collections in Europe, South America, Asia and the United States. Stig also designs logos, cards, prints and posters for various organizations and institutions. Major contributions are the life-size orchid illustrations in "Thesaurus Dracularum", and "A Treasure of Masdevallia", authored by Carlyle Luer of Sarasota, and published by Missouri Botanical Garden, St. Louis, Missouri.

Dinner Evening

There will be an evening dinner at Froggy's French Café located at 303 Greenbay Road, Highwood, IL 60040, a ten (10) minute drive from the hotel, phone number 847-433-7080. There is a choice of five entrée's to be made at the time of seating. The cost of the dinner is $40.00 per person. The total capacity available for this

dinner is 50. Reservation may be made by contacting Sue Golan. To make and hold a reservation a check must be sent to Sue Golan.

Sue Golan
E-mail Address: sgolan@aol.com
Phone Number: 842-234-6311
Fax Number: 847-234-6397
599 Old Mill Road
Lake Forest, IL 60045

In all case reservations must be followed up with payment to Sue.

A cash bar will be opened starting at 6:00PM
Dinner will be served at 7:00 PM. Following dinner will be the auction of a number of antique Odontoglossum Alliance hybrids and other plant material.

Dinner Menu

Canapés
Lobster bisque (Soup du jour may be substituted)

Green Salad
Choice of
- **Coq au vin**
- **Tournedos of beef with Roquefort sauce**
- **Slice breast of duck with cassis sauce**
- **Grilled salmon Provencal**
- **Fish du jour**

White and dark truffle cake with berries

WHAT'S IN A HYBRID?

Steve Beckendorf

Two of the articles in this newsletter have a common theme - how can you predict or find out the contributions of ancestral orchid species to any particular hybrid. The first article, from Helmut Rohrl, explores the genetic predictions if one knows the geneology of the hybrid. The second, an article by Norris Williams and Mark Whitten that was originally published in "Orchids", describes some molecular detective work to settle an argument about the parentage of an Oncidium X Tolumnia hybrid.

In the first article Helmut Rohrl constructs a mathematical model based on the distribution of chromosomes from the initial parental species into a present day hybrid. Using this model, Helmut shows that one would not expect chromosomes from a species used 2 or 3 or 4 generations ago to be proportionally represented in a particular present day hybrid. Thus the common practice of talking about a hybrid as having a certain percentage of its genes from a particular species is very misleading. The fallacy is sharpened by two additional facts.

First, the plants that we grow are not a representative sample of the genetic contributions of the two parents at any generation. For any particular cross, only a few plants (1 to a few hundred) are ever flowered, even though the cross might have produced a few hundred thousand seed. Initially, only a fraction of the seed are flasked. Only a small fraction of the germinated seed are replated, and of these only the strongest or largest are chosen to plant out into community pots. Selection for vigor often occurs several more times as the seedlings are individually potted and as they mature towards flowering size. Finally, we make a stringent selection when they flower, eliminating the small, ungainly, drab-colored, etc. As a result, the plants that are grown and used for further hybridizing are not at all like the average progeny of the cross, let alone a representative sample of the genes that entered the lineage several generations back.

For example, let's look at a single gene A. If the two plants in a cross have four slightly different varieties of A that they had inherited from their parents, we could say that the first plant has varieties A1 and A2 and the second has A3 and A4. In the progeny of the cross we would expect to get equal numbers of A1/A3, A1/A4, A2/A3 and A2/A4. But the plants that were chosen to breed on might all carry A2/A3, perhaps because this combination gave more vigorous plants, or produced a particularly pleasing dark red color, or even just because of chance. If so, A1 and A4 would never appear again in subsequent generations. Thus the species that contributed A1 and A4 several generations back in the lineage would no longer be contributing to the inheritance of A in later generations.

Second, our strongest selection is focussed on a small number of genes that affect flower morphology. Thus their distribution to the next generation will be radically skewed compared to the vast majority of genes. We know of substantially fewer than 100 genes that directly regulate flower shape, color, pattern, scent, substance - the characters used to select parents for the next generation. In contrast, there are probably 10,000-20,000 genes in each nucleus that are crucially important to development of the plant, but we don't distinguish among them as we select the next AMs and FCCs. These genes will contribute on average 50% to each plant of the next generation, but we don't notice their effects. When we talk about the contributions of a species to a particular hybrid, we are only talking about contributions of the selected few genes, and like elites everywhere, they don't follow the simple rules.

In the second article, Norris Williams and Mark Whitten show that DNA analysis can unravel the lineage of a hybrid by identifying sequences contributed by both parents and in this case by grandparents and even great grandparents. This analysis is similar to the forensic DNA analysis used to identify a murder suspect or a deadbeat dad. For example, it is possible to identify the father by comparing the child's DNA with that of its known mother and alleged father. If we look at a particular gene in a large number of people, we find many of small changes in the sequence. Thus two alleged fathers will have easily distinguished sequence differences. However, since the father and child share exact copies for half of their genes, the true father can be identified with a high degree of certainty.

Williams and Whitten, along with Mark Chase, have been applying this logic to distinguishing species within the Oncidiinae, our favorite group of orchids. Norris will give a talk about this work at our annual meeting and in part the article from Orchids is reprinted here to help prepare our members for that talk. The article introduces many important ideas, and I just want to mention a couple of things that helped me understand it.

First, the sequence that they and many other DNA taxonomists often use to distinguish among species is called the ITS (internal transcribed spacer). It was chosen, in part, because it is not expected to be strongly influenced by selection, either natural selection as Darwin and successors envisaged it, or the unnatural selection

that we provide in our greenhouses. (Actually, what we do in our greenhouses wasn't an important factor in choosing ITS for evolutionary or taxonomic studies!) If selection has little effect on the precise sequence of the ITS, we expect it to change gradually with time as a result of rare mutations that introduce one or a few sequence changes. Thus, the longer period of time that two species have been separated, the more changes we can expect to find between their ITS sequences. By comparing the sequences of many species, it is possible to identify the species that are most like each other and to group them together in an evolutionary "tree".

When one has data from a large number of species, building a tree can be very complicated, and biologists tend to use several different statistical methods (or rather their computers use these methods) to build consensus trees and to evaluate how reliable any particular branch in the tree is. As the tree becomes better established, related species tend to be grouped on adjacent branches of the tree. A group of species that are all located on twigs connected to a single branch of the tree is known as a clade. All species within the clade are more closely related to each other than to any species on another branch. For example in the tree shown in this article, the Brazilian oncidiums dasystyle, concolor, flexuosum and bicolor are members of a clade that is clearly separated from the Tolumnia clade at the top of the tree.

There is one other thing about the ITS data that may lead to some confusion. When Norris and Mark isolated the ITS sequences from a single leaf of the hybrid, they identified at least 5 distinct types, three within clone group A and two slightly different ones in clone group B. Normally we expect just two alternative forms of a gene or sequence in a diploid plant, one from the mother and one from the father. What's going on? Well, the ITS sequence is special in a second way, in addition to being relatively unaffected by selection. ITS sequences are repeated many times in each nucleus; there are clusters of repeated sequences containing the ITS on several chromosomes. Thus it is likely that a particular hybrid will have ITS sequences that it inherited from several of its ancestors, not just two as would be the case for a normal diploid gene. This serendipity allowed Norris and Mark to find sequences that are characteristic of at least 3 Tolumnias as well as Onc. flexuosum in a single hybrid plant.

In summary, these are two very interesting articles. Helmut presents a new way to analyze genetic inheritance, and Norris and Mark show how the application of modern molecular methods can solve a variety of questions that orchid growers have long puzzled over.

Oh Say, Can You See...

How Much Of A Species Is In A Hybrid ?

A Model for Inheritance in Orchid Hybridizing

Part 1

by Helmut Rohrl

The question posed above is one frequently heard in discussions among orchid enthusiasts, hybridizers, and judges when they are assessing a blooming hybrid plant. The Wildcatt database, a widely accepted reference for orchid hybrids, indicates the species ancestry of a particular hybrid as percentages. For example, Cattleya Bow Bells (Cattleya Edithae X Cattleya Susan Hye), is said to have following species composition: C. trianaei 25 %, C. gaskelliana 37.5%, and C. mossiae, 37.5 %. This type of information, while commonly used in articles, presentations and discussions, is meaningless and misleading in most cases. In this paper we will examine whether, and how well, this commonly-held perception of orchid genealogy fits current scientific understanding of inheritance in orchids. In order to accomplish that, we will split the original question into several, more specific ones.

Considering the species in a hybrid's genealogy, what is the genetic makeup of the resulting hybrid?
How, and why, do the "actual progeny" of a cross differ from the "model-based progeny"?
How do dominance and other factors influence the physical attributes of a hybrid?
What can be said about any inheritance that hybrid progeny receive from one parent only?
What role does polyploidy play in the outcome of a cross?

We will find some answers by developing a model for the genetics of orchid breeding, and by drawing logical conclusions from that model. Whenever natural phenomena are studied by means of models, these models must be simple enough to allow accurate conclusions, yet provide enough detail to obtain non-trivial and quantifiable results. It is also essential that the model be founded on assumptions that are derived from scientifically established facts.

Cytology in a Nutshell

Our model for the genetics of orchid breeding is based on cytology, which is the study of the structure, function, and reproduction of plant cells and their components.[1] Every living plant is made up of cells, each of which is surrounded by a cell wall. Inside the cell wall is the cell membrane, a separate entity which encloses the cell's cytoplasm and the cell's organelles, including the nucleus. Certain organelles, the mitochondria, are the site of respiration and metabolism, and could be thought of as the cell's engine. The most distinctive organelle in the cytoplasm is the cell nucleus. The nucleus contains chromatin, which is normally composed of pairs of threadlike bodies called chromosomes. The chromosomes consist primarily of deoxyribonucleic acid (DNA), an extremely long double helix built of cross-linked deoxynucleotide bases.

It is the precise sequence of these base pairs which codes for the unique genetic template of a plant.

Chromosomes in every normal, non-reproductive plant cell come in pairs. In plant species the chromosomes in each pair are homologous, that is, essentially identical in their genetic makeup.

The complement of chromosomes in a cell is called the cell's genome. The genomes of essentially all non-reproductive plant cells are essentially the same. While the genome is exactly the same within virtually all somatic cells of an individual plant, different genes are 'turned on' in different cells, leading to cell to cell functional differences, e.g. leaf cells develop into stomata, while root cells do not, even though they both have identical genetic material. The genome of a plant is the genome that is common to most somatic cells. In contrast to the notion of the 'genome of the cell' the notion of 'genome of a plant' is a construct. Organelles other than the nucleus can also carry some genetic information. The sum of genetic information contained in a somatic cell is the same for virtually all cells, and this common genetic information is referred to as the genotype of the plant. The genotype of a plant determines its phenotype, that is, its physical appearance and physical attributes, such as flower color, flower size, stem length, temperature tolerance, bloom season, etc.

Genes are chains of nucleotides. They are linked like beads on a necklace, the necklace being the chromosome. All enzymes and other proteins which carry out the life functions of a cell are manufactured according to the blueprint contained within the genes.

The total number of chromosomes in a somatic cell is called the somatic chromosome number of that cell. Somatic chromosome numbers in orchids range from 10 (in Oncidium pusillum) to 200 (in some Aerangis species). The most common chromosome numbers for orchid plants are 28, 38, and 42. Within the grex[2] of any given species or hybrid almost all progeny have the same somatic chromosome number, which happens to be an even number. These plants are termed diploid, and their chromosome numbers are written as 2n.Occasionally, however, cultivars are produced whose somatic chromosome number is different from the diploid number. If this number is kn, where k is a number greater than 2, then these genomes are called euploids; more specifically one has triploids (k = 3), tetraploids (k = 4), pentaploids (k = 5), hexaploids (k = 6), septaploids (k = 7), octoploids (k = 8), and so on as far as you can count numbers in Latin. A triploid cultivar of Oncidium pusillum, for example, has ten chromosome "bundles", each consisting of three homologous chromosomes (instead of ten chromosome pairs having two chromosomes each). for a total of 30 chromosomes, while a pentaploid would have five per chromosome bundle. Any euploid plant other than a diploid is called a polyploid plant, or simply a polyploid. Cultivars with a chromosome number other than a euploid number in their somatic cells are referred to as aneuploids. Aneuploids appear in certain crosses between parents of different ploidy, e.g., between a diploid and a triploid. [3]

Plants containing polyploid genomes are individuals with more than one complete set of chromosomes, and are produced in two ways. First, autopolyploidy arises from a single source, be it one plant or one cell. So, if AA represents the somatic genome of the single source, then the somatic genome of autotriploids would be AAA, by the addition of another single A chromosome to the chromosome 'bundle'. Autopolyploidy can be created in the laboratory by treating cells with colchicine, an inhibitor of cell division, but it can also result naturally (see below).

The second type is called allopolyploidy; it results when chromosomes come from two different sources. For example, if the participating parents have somatic genomes AA and BB respectively, then allotriploid progeny

would either have somatic genome AAB or ABB. Allopolyploids are obtained when crossing diploids with other allopolyploids.[4]

Somatic cells of an orchid reproduce by a process called mitosis. First the chromosome pairs are replicated to give two identical copies, then the nucleus divides, followed by the division of the other organelles and the cytoplasm. Mitosis is responsible for the plant growth, and has no direct role in our considerations of orchid breeding.

A second type of nuclear division, called meiosis, is responsible for producing gametes, the reproductive cells. In flowering plants, gametes are the pollen and egg cells depending on the anatomical location in which they are produced. During meiosis, chromosomes undergo one round of mitotic division, then go through a meiotic division to produce gametes with normally only one copy of each chromosome. The complement of all chromosomes in a gamete is called the gametic genome. In orchids the gametic genome of female and male gametes is the same.

Meiosis in 2n-diploids leads to regular n-gametes, that is, gametes whose genome consists of half of the normal chromosome number of 2n. These gametes are also called haploid cells, or simply haploids, and their chromosome complement is referred to as the haploid chromosome number.

The number of chromosomes in a gamete's nucleus is referred to as the gamete's chromosome number. Almost all gametes produced by an individual plant with somatic chromosome number k have ½ the parent's chromosome number, or k/2. However, gametes are not always haploid. In 3n-triploids, meiosis produces, among others, n-gametes as well as 2n-gametes and 3n-gametes. In addition, 4n-tetraploids produce n-gametes, 2n-gametes, 3n-gametes and 4n-gametes, while 5n-pentaploids result in n-gametes, 2n-gametes, 3n-gametes, 4n-gametes and 5n-gametes, and so on. Other gametes do not fit into this pattern. However, while almost all gametic chromosome numbers of a diploid (2n) equal n, the gametic chromosome number of a triploid (3n) will generally be n or 2n, and those of a tetraploid (4n) will usually be 2n, with n and 3n occuring occasionally.

When two gametes merge to form a zygote, or embryo, the number of chromosomes in that zygote is simply the sum of the chromosome numbers of the participating gametes. Generally for a 2n-species, the gametes must be n-haploids as can be seen by the following deduction. Hypothetically, if the gametes were to have xn chromosomes (where x is any number), then the resulting progeny would have double that number, or 2xn chromosomes. Continuing with this scenario, the next generation would have $2xxn = 2x^2n$ somatic chromosomes, the one following this one would have $2xxxn = 2x^3n$ somatic chromosomes, etc.[5] So if x were to be more than 1, chromosome numbers of progeny would explode exponentially. On the other hand, if x were less than 1, chromosome numbers of the progeny would diminish exponentially. Clearly, this scenario is not what is observed in nature. This means that stable breeding behavior almost always occurs only for gametes with chromosome number x = 1, that is, for haploids.

By assigning reference numbers to the pairs of chromosomes in the somatic cells of a species, we can refer to individual chromosomes, such as #3 or #25. They can generally be distinguished from each other when stained and viewed under a microscope. The way in which chromosomes are assigned reference numbers is arbitrary, although it is generally done according to chromosome size. The chromosomes in gametes of a par-

ticular species acquire the same 'reference' number as the chromosome pair from which they were derived. In other words, gametic chromosome #4 comes from chromosome pair #4 in the parent cell. Chromosomes with the same number are, for our purposes, considered homologous, that is identical, in their base sequence and structure in virtually all individual plants of a given species. For instance, chromosome #4 in L. anceps 'Rio Bravo' is considered to be identical in its genetic code, or genotype, to chromosome #4 in L. anceps 'SanBar'. In fact, differences between the whole genomes of cultivars of two entirely different species can be miniscule. The genomes of man and his closest relative, the chimpanzee, are about 98.5 % identical.

When crossing two cultivars of the same species, each chromosome pair in the zygotes of the progeny is built from the gametes' chromosomes with the same reference number. This fact is often referred to as the independent assortment of chromosomes.

The basic laws of genetics were first discovered and published in an obscure local journal in 1865 by Gregor Johann Mendel. His work was quickly forgotten but was rediscovered in 1900. In 1897 C.C.Hurst proposed that the chromosomes play a significant role in heredity. His subsequent investigations were based on Mendel's laws. W.S.Sutton conjectured in 1902 / 1903 that chromosomes and the mendelian laws of genetics were interconnected. The double helix structure of the chromosome pairs and the importance of DNA in heredity were established by F.H.C.Crick and J.D.Watson in 1953.

A Model For Mendelian Inheritance in Orchids

Mendelian inheritance in plants is the genetic legacy transmitted by chromosomes of the gametes: pollen and egg cell. To visualize how this inheritance occurs, we will develop a model describing what takes place during sexual reproduction in orchids, as the chromosomes are carried from gamete to zygote. Like all models, it is designed to approximate reality, yet to be simplified enough so we can draw conclusions without having to account for irregularities.

Using what is known about orchid cells, we make four central assumptions in creating our model for the genetics of orchid breeding.

1. In all individual plants of an orchid species chromosomes with the same chromosome reference number are considered homologous (i.e., identical).
This means that the two chromosomes in chromosome pair #4 of L.anceps 'Riviera' are assumed to be identical with each other, as well as with the chromosomes in chromosome pair #4 of L.anceps 'Splish Splash'.

2. Every somatic cell that generates gametes is a 2n-diploid.
Our model does not immediately deal with triploids, tetraploids, and other polyploids; we will adapt our model for polyploids later on.[6]

3. Every gametic genome is 'regular', that is, 1n-haploid.
Later on we will discuss the role of irregular gametes, that is, gametes with numbers of chromosomes other than 1n in our model.

4. In a zygote, each chromosome pair is the combination of gametic chromosomes with the same chromosome reference number.
We assume that during the formation of a zygote, chromosome #6 from the pod parent's gamete matches up with chromosome #6 from the pollen parent's gamete. The model can be modified to account for mismatched chromosome pairs in zygotes.

These assumptions allow us to describe the hypothetical progeny of a cross between two orchid cultivars. First, for a given cultivar, we must list the chromosome pairs of its genotype, and all its possible gametes, in terms of the species in their ancestry. Because chromosomes sort independently during meiosis, gamete genomes can have a variety of combinations of chromosomes in each pair. For example, if chromosome pair #1 of the cultivar comprises one chromosome from species S_1 (e.g., a parent) and one from species S_2 (e.g., a great grandparent), then we can write S_1 S_2 for the cultivar's first chromosome pair. Similarly for chromosome pair #2: if one chromosome came from species S_3 ,and the other chromosome from species S_1, then chromosome pair #2 appears in the list as S_1 S_3, and so on. It is unwieldy to write botanical names for the species source of all chromosome pairs, so we will use symbols such as letters A, b, ..., or other symbols such as >, /, |, to represent different chromosomes, and enclose the list of chromosome pairs in brackets. So, in our example, the cultivar's genotype can be represented as [S_1 S_2, S_1 S_3, ...] or, if we choose / for S_1, \ for S_2, and ≈ for S_3, the gentoype can be represented as [/ \, / ≈,...]. The pattern of symbols for all chromosome pairs thus represents the genotype of that cultivar.

This symbol representation allows us to show the parental (or grand-parental, etc.) source of each chromosome in each chromosome pair. Cultivars from entirely different crosses can have identical symbol patterns, even though the species ancestry used in the cross is different. For example, the primary cross Oncidium tigrinum x Brassia verrucosa can be described by [AB, AB, ...], where for each chromosome pair AB, chromosome A came from Onc. tigrinum, and B from Brs. verrucosa, for example. The primary grex Phalaenopsis equestris x Vanda coerulea will have an identical chromosome pattern, using symbols [|\, |\, ...], or if you prefer letters, [AB, AB, ...],. On the other hand, the simple primary cross of Onc.tigrinum x Brs.Rex, (Brs.Rex is Brs.gireoudiana x Brs.verrucosa), results in cultivars such as [AB, AC, AB, ...] which are not similar to any cultivar in the primary grex Onc.tigrinum x Brs.verrucosa. However, due to independent chromosome assortment, Onc.tigrinum x Brs.Rex actually yields some cultivars that are identical to those in Onc.tigrinum x Brs.verrucosa, namely [AB, AB, ...] and [AC, AC, ...]. When we replace the symbols with actual species names, the hypothetical progeny for the Oncidium tigrinum x Brs. Rex include both the primary hybrids Onc.tigrinum x Brs.verrucosa and Onc.tigrinum x Brs.gireoudiana.

To summarize:

A species' somatic genotype can be symbolically represented as:

one pair of chromosomes:	[||] or [JJ], or similar
two pairs of chromosomes:	[||, ||], or [JJ, JJ], or similar
n pairs of chromosomes:	[||, ||...,||], or [JJ, JJ, ...,JJ]

Note that the pairs of chromosomes in each species are homologous, or identical.
Correspondingly, a species' gametic genotype can be written as follows.

one chromosome:	[|], or [J], or similar
two chromosomes:	[|, |], or [J, J], or similar
n chromosomes:	[\, \,...\ ] or [R, R,...R]

And a hybrid's somatic genotype can be symbolically represented as:

One pair of somatic chromosomes:	[/ |], or [DP], or similar
two pairs of somatic chromosomes:	[/|, \\|], or [YF, ND], or similar
n pairs of somatic chromosomes:	[> /, + =,...,+\ ], or [ηδ, θθ,..., ϕβ]

Note that the pairs of chromosomes in a hybrid cultivar, may, or may not be homologous, i.e., not identical to each other.

For simplicity, let's first consider the cross of an orchid species with just one pair of chromosomes, and so, just two possible gametes. We'll call one plant ♣ (the pod parent with genotype [\|\|]), and the other ♦, (the pollen parent with genotype [//]). The cross between those two plants is usually written as ♣ x ♦. As described earlier, 1n gametic genomes result when chromosome pairs in the nucleus separate into single chromosomes. In our model, we will represent gametic genomes by selecting one symbol from each chromosome pair in the parents' somatic genome, then writing down all possible combinations for progeny.

As shown below, along the top we write the two possible gametes coming from parent ♦, that is, [/] and [/] . On the left we write the two possible gametes coming from parent ♣, which are [\|] and [\|]. Combining one gamete from the pollen parent with one from the pod parent, we obtain zygotes with genotype [\|/]. The 2 x 2 array obtained in this manner is called the zygote matrix of the cross ♣ x ♦, and shows all possible outcomes resulting from this grex with one chromosome pair. The zygote matrix in this case appears in bold font. The zygote matrix for grex ♣ x ♦ is:

		Parent ♦ gametes	
		[/]	[/]
Parent ♣ gametes	[|]	[|/]	[|/]
	[|]	[|/]	[|/]

The probability that a particular individual will appear in the progeny population is equal to the number of times it shows up in the zygote matrix, divided by 4. For example, if a particular genetic combination appears twice in the above zygote matrix, half of the progeny of that cross will be AB (2 appearances in the matrix divided by 4 possible appearances = 50%).

It is important to note that zygote matrix configurations reflect only the genotypes of progeny populations and their probability distributions The matrix cannot reveal the phenotype of progeny populations, i.e., it cannot tell us what the physical appearance and attributes of the various cultivars will be. To know what each cultivar will look like would require us to know: which chromosomes the hybrid plant has inherited from its ancestors; which genes are on each chromosome; and what each of those genes codes for, such as stem length, flower size, etc. At this time, we do not have the capability to perform these kinds of analyses, so it is not

possible to predict phenotype on the basis of genotype.

Here are definitions for hybridizing terms we will be using:

simple primary hybrid	species x species
complex primary hybrid	species x hybrid - or - hybrid x species
complex hybrid	hybrid x hybrid.

Now we are ready to construct the zygote matrix for seven basic crosses, which include simple primary hybrids, complex primary hybrids, and complex hybrids. In the zygote matrix, the progeny are shown in bold type.

In the examples below, symbols within the brackets represent the genotype, not particular chromosomes. A species is represented by a sequence of homologous symbols , i.e., [DD,...,DD] while hybrid cultivars may be represented by both homologous and heterologous symbols, i.e. [KQ .... BB].

[1] An overview of plant cytology can be found in [A1], [EB], as well as in [SCH] and [W]. Details on genetics can be found in the textbook [H].

[2] GREX DEFINITION IS MISSING

[3] A list of somatic chromosome numbers for selected orchids can be found in the Appendix of [A2].

[4] Allotetraploids with genome AABB are called amphidiploids or double diploids; when used in hybridizing, they behave like diploids.

[5] The symbol x^2 is used for the product xx, x^3 for the product xxx, and x^k for the product of k copies of x.

[6] See section on Polyploidy

(This is Part 1 of Helmuts article. The balance of the article will be printed in subsequent Newsletters)

Editors Note:

With permission of The American Orchid Society and the Authors we are re-printing the article "Checking an Orchid Hybrid's Background" by Norris H. Williams and W. Mark Whitten. This material while interesting and scientificlly important, will also serve as background for the two afternoon lectures on taxonomy to be given by Norris Williams and Stig Dalstrom at the 12 April 2002 Odontoglossum Alliance meeting. The advances in molecular technology, so prevalent in today's world, have pioneering aspects to our world of orchids. Professor Williams is a leading researcher in the use of this technology for the understanding of plant relationships.

Checking an Orchid Hybrids Background

The use of molecular data in determining parentage of hybrids

By Norris H. Williams and W. Mark Whitten

For several years we have used DNA sequencing to help clarify the evolutionary relationships among orchid species. For orchid taxonomists, this exciting work is an end unto itself. Orchid enthusiasts who are less interested in the arcane details of classification have often asked us whether this sort of research has any application to the community of orchid growers and hybridizers. In this article, we present some recent results that show one of the practical uses of DNA sequencing to horticulturists.

For some time, there have been heated discussions concerning the validity of certain hybrids. In some cases, opinions differ about whether a certain plant is a "pure" species or it might have some parentage from another species. In other cases, the exact parentage of a horticultural hybrid is disputed. These controversies are based on the overall appearance of the flowers and plants, and by our expectations of traits imparted by the putative parents. Experienced hybridizers can easily identify the parentage of many primary and some advanced hybrids, but such expertise is subjective and open to dispute. The question of how to accurately and positively identify hybrids, their parents, and pure species has been approached in the past by using various morphometric techniques. These techniques, such as calculating hybrid indices based on measurements of flower parts, have been less than satisfactory. With the advent of automated DNA sequencing, sequence data and fingerprinting techniques should enable us to obtain more objective answers to these problems.

The example we present here deals with the true identity of a hybrid between *Tolumnia*and *Oncidium.* In recent Internet discussions, there was a debate on the true parentage of the purported hybrid *Oncidium* (syn. *Tolumnia*) Golden Sunset x *Oncidium flexuosum* grown by Bruce Ritter (http://members.aol.com/ntropics/Onc.htm). *Oncidium* Golden Sunset is a complex hybrid utilizing several species of equitant oncidiums, which are also known under the genus name *Tolumnia (Tolumnia triquetra, Tolumnia pulchella, Tolumnia urophylla* and *Tolumnia guianensis* [syn. *Desertorum]). Oncidium flexuosum* is a member of a Brazilian group of oncidiums, including such members as *Oncidium bicolor* and *Oncidium concolor*. The purported hybrid plant certainly looks much like a hybrid *Tolumnia* dominated by *T. triquetra*, and it was questioned whether there indeed was any *Onc. flexuosum* genetic material in this plant. A discussion-group member commented that perhaps DNA testing could help solve this question of disputed paternity, and we decided to explore the situation.

We extracted DNA from a leaf of the purported hybrid plant and amplified the ITS 1 and 2 (Internal Transcribed Space) region from the total DNA. This region is commonly used for plant systematic studies, including orchids (Cox et al., 1997; Whitten, Williams and Chase, 2000). Because a hybrid plant would contain a mixture of ITS types (one or more from all of the ancestors), we cloned the DNA product into plasmids in bacterial colonies, which allows us to separate the different ITS types (only one ITS copy is taken up by each bacterium). We then reamplified and sequenced the ITS region from a dozen random bacterial colonies ("cloned" DNA), and compared the resulting sequences with our data base of about 460 spices of Oncidiinae. Out of the 12 cloned ITS copies from the hybrid, four were nearly identical to *Onc. flexuosum*. The other eight are similar or identical to various *Tolumnia.*

The entire aligned ITS data matrix is approximately 735 bases long. Figure 1 (See page 20) shows a small portion of the entire ITS sequence matrix for various species of *Tolumnia,* the *Onc. flexuosum* complex, and sequences from the cloned DNA's of the purported hybrid. We show here just a 32-base portion to illustrate how powerful this technique is in resolving this type of problem. For example, note positions 4 and 32, where all the *Tolumnias* have a "T" and the *Onc. flexuosum* group have a "G". At positions 20-22, all *Tolumnia* species (and the Clone Group A) share a three-base deletion that is not present in the *Onc. flexuosum* group.

In just this 32-base example, there are 14 diagnostic sites. At least 46 sites in the entire 735-base matrix are diagnostic and show the hybrid nature of the plant and the relationship of the hybrid to the parental types. The important things to note are the number of sites in which Clone Group A matches with the *Tolumnia* species, and how many sites that Clone Group B matches with *Onc. flexuosum*. Four of the Clone group A sequences associate with *T. urophylla*, three with *T. triquertra* and the *T. pulchella* group, and one with the unresolved group of *Tolumnia* species (Figure 2 See page 17). Three of the Clone Group B sequences associate with the *Onc. flexuosum*, and the fourth sequence is slightly divergent, but still most closely associated with *Onc. flexuosum*. We know that *T. pulchella, T. triquetra, T. guianensis* and *T. urophylla* are all in the parentage of *Onc.* Golden Sunset, so these data agree well with our expected results. Unfortunately, the group of *Tolumnia* species that includes *T. triquetra, T. guianensis, T. pulchella* and *T. urophylla* have ITS sequences that are very similar to each other, and it is not possible to completely distinguish among potential *Tolumnia* parents based solely on ITS data.

Figure 2 shows the results of a complete analysis of the entire 735 bases. This graphically demonstrates the Clone Group A DNA's are related to various *Tolumnia* species, and the Clone Group B DNAs are related to *Onc. flexuosum*.

The DNA evidence clearly shows that this plant is an intergeneric hybrid involving several species of *Tolumnia* and also a plant similar or identical to *Onc. flexuosum*. So, we conclude that *Tolumnia* can be hybridized with non-*Tolumnia oncidiums*. Whether this hybrid is sterile is more a matter of ploidy level and chromosome pairing, and is a somewhat relative concept that depends on the persistence and skill of the breeder. A more general conclusion is that gross floral morphology is not a good indicator of parentage or relationships among these orchids (and probably many others). The concept of one parent dominating appearance of off-spring is well-established by orchid hybridizers. Finally, we should note that *Onc. flexuosum* is a member of a group or clade of predominately Brazilian species that includes *Oncidium bicolor, Oncidium dastyle* and *Oncidium concolor*. Phylogenetically, these are very distinct from the group that contains the type species of *Oncidium (Oncidium altissimum)*. Until the classification and nomenclature of the Oncidiinae is revised (which is under way, based in part on molecular data), asking, "Can you cross a *Tolumnia* with an *Oncidium*?" will remain a trick question. The answer depends on which of many of diverse "Oncidiums" you are talking about. But we do have the resources now to answer many such questions.

Future Applications

The use of DNA sequencing to settle this relatively trivial dispute among friendly growers might seem a case of technological overkill. However, it provided the perfect example to demonstrate the potential power of these techniques. Using the same applications, we should be able to settle questions of hybridity for clones of intergenerics and interspecific hybrids, as well as putatively pure species, such as *Pragmipedium schlimii, Paphiopedilum sanderianum, Zygopetalum mackayi* and many other taxa. Some caveats are in order.

This technique cannot distinguish among closely related species that have similar or identical ITS sequences (e.g., some *Tolumnia*; many *Cattleya* species). More sensitive techniques such as AFLP™ (Amplified Fragment Length Polymorphism) exist that are capable of distinguishing such species, and even providing a unique fingerprint for a selected clone or cultivar. Many other orchids, such as *Paphiopedilum* and *Phragmipedilum*, exhibit species-specific ITS sequences, and these techniques should be admirably suited for answering questions about the parentage of primary and complex hybrids. For example, one grower approached us with a batch of unflowered *Paphiopedilum* seedlings he had purchased as a sib cross of a rare species. Based on the seedling morphology, he suspected that they were actually interspecific hybrids and not the pure species. Sequencing of the cloned ITS region from the unflowered seedlings should easily determine their parentage, since a large database of *Paphiopedilum* sequences already exists (Cox et al., 1997). Another caution is that the evolution and behavior of the ITS region in hybrids is not well studied. It is possible that

recombination might give rise to hybrid ITS types that are intermediate or distinct from the parental types. Until we examine more hybrids using these techniques, their limitations and potential will not be fully known.

Another example of the potential utility of DNA data concerns the Ecuadorian *Odontoglossum edwardii*, whose vivid purple flowers are highly valued for their hybridizing potential. Although originally described as an *Odontoglossum,* it has been treated as an *Oncidium* or a *Cyrtochilum*, depending on taxonomic whims (reviewed by Withner, 1994) Several years ago, Howard Liebman (1998) pointed out that *Odm. edwardii* failed to cross readily with various species of *Cyrtochilum* (e.g. *Cyrtochilum macranthum).* Simultaneously, we obtained ITS sequencing data for *Odm. edwardii*, which clearly placed it as a *Cyrtochilum*, not with *Odontoglossum*,. The evolutionary trees based on sequence data (and the resulting classification scheme) should have considerable utility to hybridizers, who could use it as a map to guide hybridization efforts. These sequence data do not reflect ploidy levels that can greatly influence the fertility of offspring, but (in general) closely related taxa should be more easily crossed than those that are distantly related.

The tools are now at hand for settling a number of controversies involving parents, hybrids and possible hybrids using DNA sequences and other modern molecular techniques. So far, no one has applied these modern techniques to orchids. By developing these applications further, and expanding the database of sequences of a large number of species, we should be able to resolve many problems involved with hybridizing orchids and settling questions of paternity. To do so, we need to increase sequencing for a large number of species and expand the number of genes used in sequencing.

Acknowledgments

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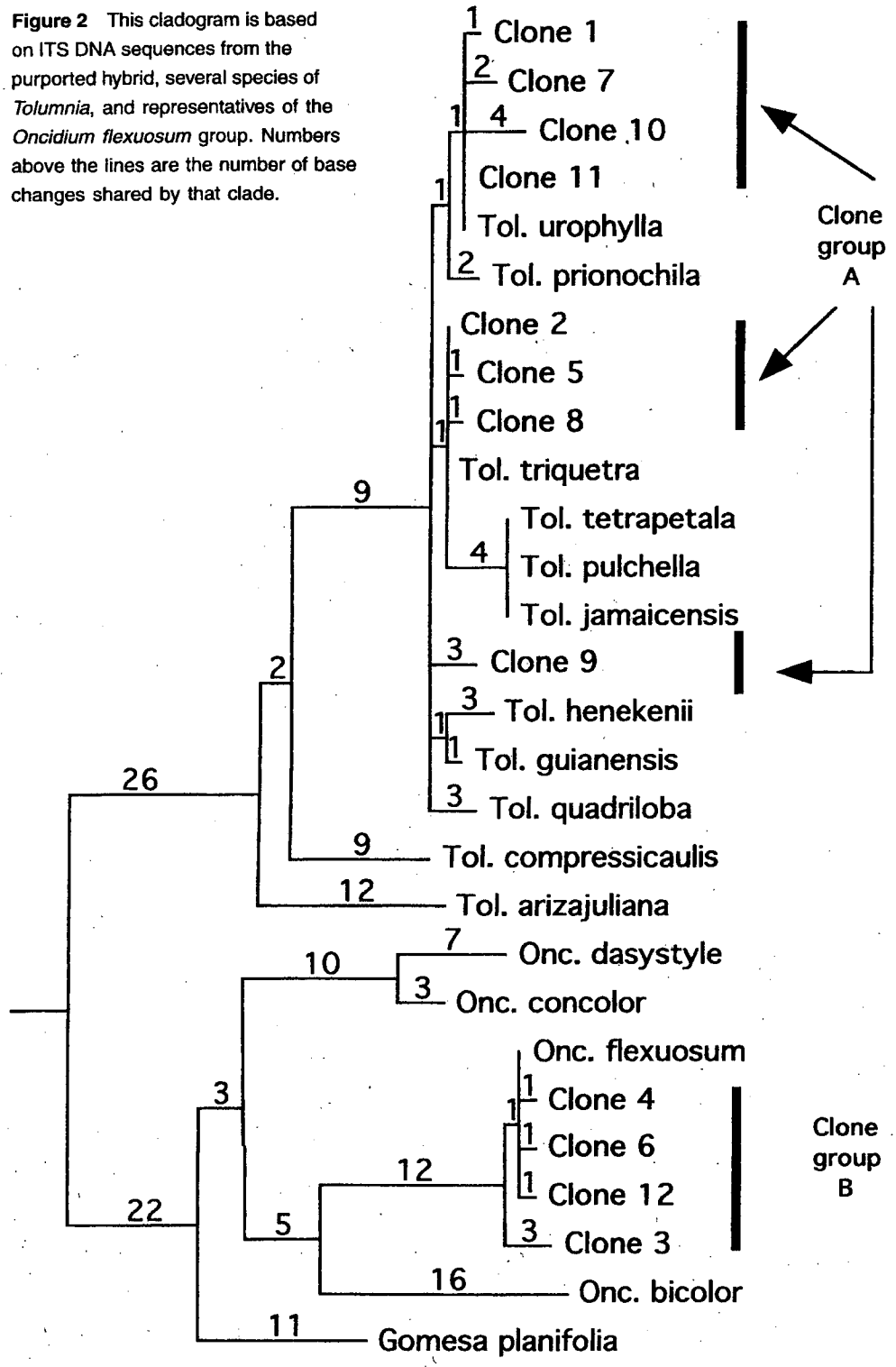
Norris H. Williams, PhD, *is curator of vascular plants at the University of Florida, Florida Museum of Natural History Herbarium, and has worked on floral fragrances, pollination biology, and the systematics and evolution of orchids. He is currently spending most of his time on a study of the molecular systematics of the Oncidiinae, funded in part by the American Orchid Society.* **W. Mark Whitten,** *PhD is also at the University of Florida Herbarium and has also worked on floral fragrances, pollination biology, and systematics and evolution of orchids. Florida Museum of Natural history, University of Florida, Gainesville, Florida 32611-7800 (e-mail orchid@flmnh.ufl.edu or whitteen@flmnh.ufl.edu).*

Figure 2 This cladogram is based on ITS DNA sequences from the purported hybrid, several species of *Tolumnia*, and representatives of the *Oncidium flexuosum* group. Numbers above the lines are the number of base changes shared by that clade.



Auction Plants

The plant auction to be held at the dinner following the Odontoglossum Alliance meeting is specializing in a number of antique odontoglossum hybrids. This will not be the onlymaeial offered at the auction. Listed below are a nnumber of the contributions that will be available at the auction. If you have material to donate antique or otherwise please bring it to the dinner or to the lecture meeting.

Lee Alyanakian's article on "The Orchids of Longwood gardens" (Odontoglossum Alliance Newsletter, August 2001) lists a number of the very early Odm and Oda. Hybrids. Lee has graciously donated to the Odontoglossum Alliance a number of divisions of some of these plants. The divisions donated are:

L1694 Odontioda Vesta registered in 1921 by Charlesworth and Company (Oda. Charlesworthii x Odm. Prince Albert)

L1685 Odontioda Naralda, registered in 1921 by Charlesworth and Company (Oda. Bradshawiae x Odm. Doris)

591454 Odm. G. (Odm. Crispum x Odm. Talluha) may not be registered.

L1689 Odontioda Red Riding Hood, Registered by F. M. Ogilvie, The Shrubbery, Oxford in 1913

940314 Odontoglossum hortensiae, This is now Rhyncostele, formerly Lemboglossum, similar to cordatum and is an intermediate grower preferring warmer temperatures than cordatum and is found in Costa Rica.

971188 Odontioda Keighleyensis registered in 1908 by Charlesworth and Company (Cda. Noezliana x Odm. Cirrhosum)

These plants will be auctioned at the Odontoglossum Alliance meeting and dinner schedule for 4 April 2002. The program for this meeting is in this newsletter.

The Odontoglossum Alliance has a number of members who have generously contributed plants to Longwood gardens in exchange for Lee's generous act.

Speaking of the auction this is the time for our members to start putting aside divisions of fine material, seedlings, and flasks for donation to the auction. The auction provides the resources for your Alliance to have interesting and exciting meetings with prominent speakers. It also provides he resources to enhance the newsletter, particularly to have the color pages included with each issue and to conduct special programs to encourage the growing of the Odontoglossum alliance material.

We have had some more generous contributions for the antique auction to be held at the Odontoglossum Alliance dinner on 12 April 2002.

From Bob Hamilton

Oda. Zephyr = Cda.Noezliana X Odm. Wilckeanum (R.G. Thwaites 1911)

Oda. Cooksonae = Cda. Noezliana X Odm. Ardentissimum (N.C. Cookson 1909)
Oda. Charlesworthii = Cda. Noezliana X Odm. harryanum (Charlesworth 1908)
Oda. Brackenhurst = Oda. Charlesworthii X Odm. Eximum (J. Gurney Fowler 1914)
Oda. Picasso 'Rubris' = Oda. Ariiea X Cda. Noezliana (Vacherot & LeCoufle 1973)
Oda. Red Flame = Grenadier X Lambeauianum (Armstrong & Brown 1937)

From Tim Brydon

Odm. Ascania 'Jester' = Antinous X Georgius Rex (Charlesworth 1925)
Odm. Quistrum 'Lyoth Angela' FCC/RHS = Nubia X Pescatorei (Charlesworth 1938)
Oda. Chargia 'Victor' = Argia X Charlesworthii (Charlesworth 1943)
Oda. Bradshawiae = Cda. Noezliana X Odm. crispum (Charlesworth 1907)

From John Miller

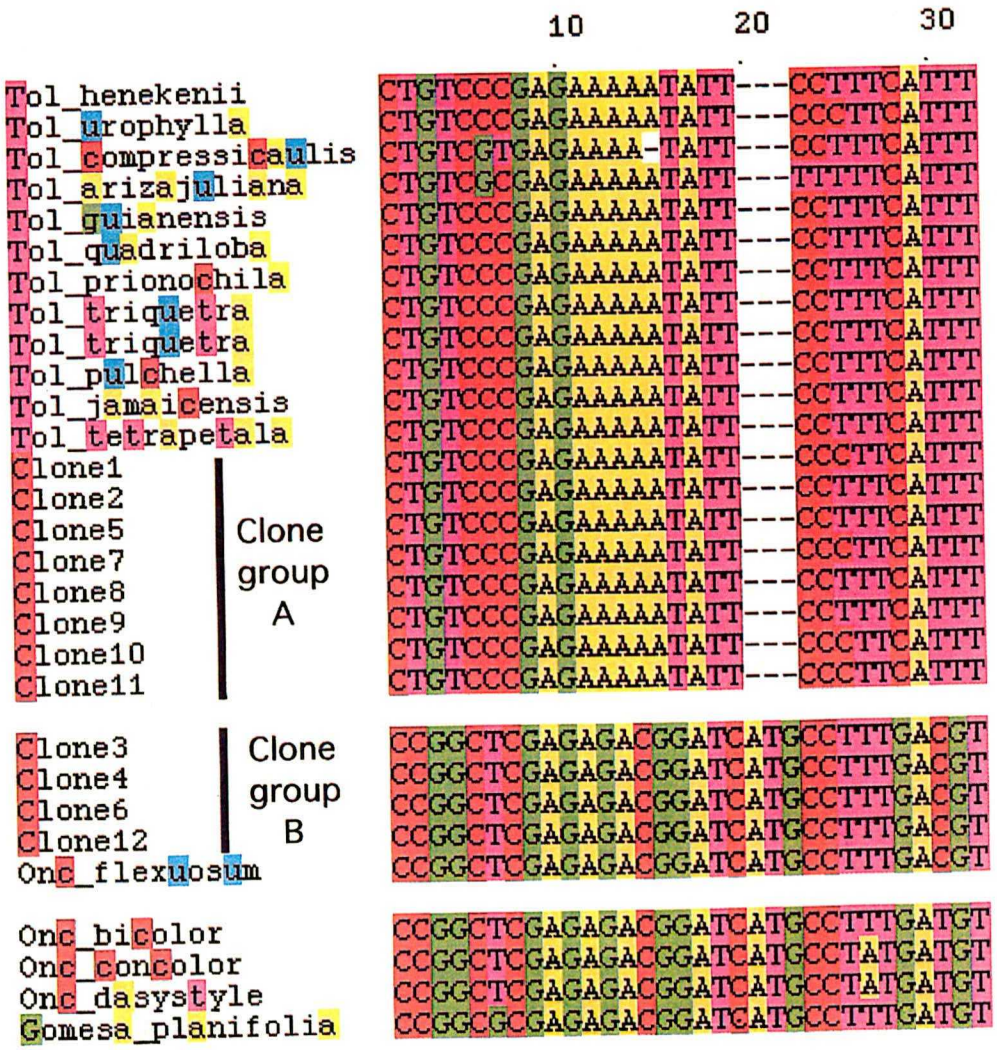
Oda. Arlington = Chanticleer X Grenadier (Sherman Adams 1937)

New Zealand Odontoglossum Alliance Newsletter

The last New Zeland Odontoglossum Alliance Newsletter received was May 2001. This newsletter contained only material reprinted from previously published newsletters. Therefore I did not reproduce it and send it out. I have suggested that New Zealand send us their material and we will publish it in our newsletter. They can, in turn, reproduce our letter for their members. We are awaiting a reply. In the meantime we will not be sending out the New Zealand newsletter, unless we receive material that warrants reproduction and mailing.

Oncidium flexuosum

Figure 1
A 32-base portion of sequence of cloned DNA from the purported hybrid and its parent species. The Clone Group A from the hybrid matches DNA sequences from *Tolumnia*; Clone Group B from the hybrid matches sequences from the *Oncidium flexuosum* group.



The two parents on the hybrid examined are *Oncidium flexuosum* page 20) and *Oncidium* Golden Sunset (opposite). Santa Barbara Orchid Estate grew this clone of *Onc. flexuosum*, 'Sana Barbara', CBR/AOS, while Malcolm and Carolyn Siegel grew the Onc. Golden Sunset, 'Malcolm' AM/AOS.

Psychopsis versteegiana

Psygmorchis glossomystax