The 2016 Banks Memorial Lecture: Cytogenetics and ornamental plant breeding: An ongoing partnership

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Abstract
Successful plant breeding requires the development of new gene combinations (genotypes) that give rise to novel characters and new cultivars that can then be tested in the marketplace. Hybridization between domesticated and wild relative(s) can provide such variation but much depends on the ease of crossing the species and to an extent their genetic and chromosomal similarity. Knowledge of chromosome number, structure and behaviour is therefore a key component of those breeding programmes that aim to widen the gene pool of existing selections. Examples taken from a variety of ornamental crops, namely sweet pea (*Lathyrus*), clivia, dahlia and pinks (*Dianthus*) will be used in this article to illustrate this important component of cultivar development.

Introduction
Plant breeding has a venerable history going back to the initial domestication of plants for food by early hunter-gatherer communities approximately ten to twelve thousand years ago. The seeds from the best plants were saved for growing in subsequent generations and as a result the genetic composition of populations of the new crop changed from those in the wild. This process has over time resulted in the reduction of genetic variation in crops, making them more uniform. Ornamental plants have probably not suffered such extreme selection, but cultivars of some ornamental genera are based on a limited number of species (or using narrow within species variation) in cultivation. Hence, existing material in cultivation may have been thoroughly exploited by amateur breeders, leaving insufficient variation to produce something new. It is also common that many useful or desirable characters, and the corresponding gene(s) that control them, are missing from the gene pool of a crop. One way to try and remedy this situation is to look for the desired characters in wild relatives and to then try and incorporate these into the plant by means of interspecific hybridization (Murray, 2003). The progeny of these hybrids have the potential to generate massive amounts of variation (as illustrated in the example in Fig. 1). However, the ability to hybridize different species is not always easy or straightforward and often an understanding of the genetic make-up of the plant and its wild relatives can aid the process of hybridization. This is where cytogenetics, the study of inheritance in relation to the number, structure and function of chromosomes, can be of value. The appearance of the chromosomes at metaphase of mitosis is called the karyotype; it originally described the number, size and shape of the members of a chromosome complement using uniform staining. In addition to uniform staining to observe shape and size, differential staining and 'chromosome painting' with DNA probes can be used to identify similarities and differences between the chromosome complements of different species (Vosa, 1985; Datson and Murray, 2003).

Fig. 1 Variation in flower form and colour in an F_2 population derived from a cross between the large flowered *Lathyrus odoratus* (top left) and the smaller flowered *L. hirsutus* (top right).

In plant breeding the setting of objectives is of primary importance. In food crops the main objectives are usually improving yield and quality but in ornamentals there is more latitude in breeding for a range of aesthetic qualities. Although there are usually clear aims when breeding ornamental plants, there can often be serendipitous events that may lead to new cultivar development. This will be seen in some of the examples that I will use to illustrate this article.

Karyotypes and ease of hybridization
Three examples from my work with Keith Hammett and our collaborators will be used to illustrate karyotypes and hybridization.

In the first, the quest for a yellow flowered sweet pea (*Lathyrus odoratus*), karyotype similarity was a key indicator of crossability. The genus *Lathyrus* contains more than 150 species and only about half a dozen of these have yellow flowers. We undertook a hybridization programme over several years using four of them, *L. annuus*, *L. chloranthus*, *L. chrysanthus* and *L. hierosolymitanus*, but these would not cross with *L. odoratus*. However, we did successfully cross many of the yellow flowered species with each other (Murray and Hammett, 1989; Hammett et al., 1996). The key indicator of crossability appeared to be a similarity in karyotype. Although all the *Lathyrus* species we worked with had the same chromosome number (diploids with $2n = 14$), the four yellow-flowered species are karyotypically quite distinct from *L. odoratus*. Pairs of them, such as *L. annuus* and *L. hierosolymitanus*, had very similar karyotypes and could be readily hybridized. In 1988 a new yellow-flowered species of *Lathyrus*,

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L. belinensis, was described (Maxted and Goyder, 1988). We obtained seed of this species and determined that its karyotype was very similar to that of the sweet pea. Although crossing was not easy and embryo rescue had to be used initially, a small number of hybrids with the sweet pea were produced (Hammett et al., 1994). Unfortunately the primary hybrids showed no evidence of yellow pigmentation (Fig. 2) and showed a similar flower colour to that of wild type *L. odoratus*. This is an example of genetic complementation where mutations in genes of one species are complemented by active alleles in the other thus confounding the initial breeding goal. However, this was not a futile exercise and is a good example of the serendipitous outcome of interspecific hybridization. Out of this programme we are yet to breed a pure yellow-flowered sweet pea but the effort has not been wasted as a significant range of new flower colours have arisen in the backcross progeny of the initial hybrid to cultivars of sweet pea (Fig. 3A–E). Several new cultivars have now been released that contain genes from that initial cross with *L. belinensis*, and these are now collectively recognized as *L.* × *hammettii.*

Fig. 2 Dissected flowers of *Lathyrus belinensis* (top), *L. odoratus* 'Mrs Collier' (bottom) and an F_1 hybrid (middle).

Fig. 3A–E A selection of sweet pea accessions showing the range of new flower colours obtained from the initial F_1 hybrid between *Lathyrus odoratus* and *L. belinensis*.

Clivia, unlike *Lathyrus*, is a genus of few species and when we started our work with them just four had been described, *C. caulescens*, *C. gardenii*, *C. miniata* and *C. nobilis*. In *Clivia*, our breeding goal was to produce a wider range of flower types and a spread of flowering times over the whole year. All species of *Clivia* have the same chromosome number (2*n* = 22), and we found that the four species we worked with all have the same basic, solid stained karyotype. However, as will be seen below, there are subtle differences between them that can be revealed using different techniques, and these demonstrate that all the species have unique karyotypes. Like in *Lathyrus*, the similarity in karyotype is an indicator of crossability and hybrids can be made readily between the four species. Many of these hybrids have interesting new flower types and show a wide range of flowering times; indeed different selections of clivia have now been produced so that plants are available that flower at all times of year.

In contrast, having different chromosome numbers is not always a barrier to interspecific hybridization. In the genus *Dahlia*, interesting hybrids have been produced at low frequencies between species with the same chromosome number, such as crosses between the tree dahlia *D. apiculata* and the herbaceous *D. coccinea* (both with 2*n* = 32 chromosomes) that resulted in plants with the tree dahlia stature and new flower colours (Fig. 4A–D). However, hybrids were also produced between *D. merckii*, with 2*n* = 36 chromosomes and *D. dissecta* with 2*n* = 34 chromosomes but not between the diploid and polyploid races within the several species where these occur (Gatt et al., 1999).

Fig. 4A–D Unusual 'tree' dahlias: hybrids between *Dahlia apiculata* and *D. coccinea*.

Manipulating chromosome numbers artificially

Garden pinks (*Dianthus plumarius*) have flowers that range in a wide variety of shades of pink through to white but there are no yellow cultivars. Elsewhere in the genus, there is one wild yellow-flowered *Dianthus* species, *D. knappii*, and there are yellow flowered cultivars of carnation (*D. caryophyllus*). We have used a white flowered pink (*D. plumarius* 'Far North') in crosses with both. Hybrids between *D. plumarius* 'Far North' with both *D. knappii* and *D. caryophyllus* were produced despite *D. plumarius* being hexaploid with 2*n* = 90 chromosomes and the other two species being diploid with 2*n* = 30 chromosomes. All progeny of crosses between yellow carnations and *D. plumarius* had pink flowers, but those from crosses with *D. knappii* were pale cream-yellow with variation in intensity between different progeny plants (Fig. 5A–B). Chromosome analysis of these variable progeny showed that the ones with the least intense yellow flowers were tetraploid with 45 chromosomes from *D. plumarius* and 15 from *D. knappii* and those with more intense yellow flowers were pentaploid with 45 chromosomes from *D. plumarius* and 30 from *D. knappii*. Thus the larger number of *D. knappii* genomes the more yellow the flowers. Where have the extra chromosomes come from? Rare chromosome mutation events can give rise to diploid rather than haploid gametes in many species including *D. knappii* which accounts for the extra chromosomes in the pentaploid hybrids. Analysis of the flower pigments from the various crosses showed that the yellow flower colour in *D. knappii* resulted from the presence of high levels of flavone and flavonol glycosides whereas those of yellow carnations were chalcones. Thus, the F_1 hybrids with *D. knappii* were yellow because they contained the same pigments as *D. knappii* but the hybrids with the carnations were pink due to *D. knappii* having the necessary genes to convert the chalcones in yellow carnations into pink anthocyanins. This is another example of genetic complementation similar to that found in the sweet pea example above.

Fig. 5 *Dianthus plumarius* 'Far North' (top left), *D. knappii* (top right) and the two types of F_1 hybrid. **A**, tetraploid hybrid (bottom; 2*n* = 60). **B**, pentaploid hybrid (bottom; 2*n* = 75).

Our pentaploid hybrids with 75 chromosomes cannot produce viable pollen and eggs so are a plant breeding dead end! What is needed are hexaploid plants of *D*. *knappii* that can be used to cross with hexaploid pinks. We are now trying to produce hexaploid plants of *D. knappii* by inducing tetraploidy using colchicine in normally diploid *D. knappii*. This has now been achieved and the tetraploid plants when they flower will be backcrossed to their diploid progenitor to produce triploids. If this is successful the triploids will then be treated with colchicine to double their chromosome number to the hexaploid level. Finally, hexaploid *D. knappii* will be crossed to hexaploid *D. plumarius.*

Despite the examples from *Lathyrus* and *Clivia*, having the same chromosome number and karyotype is by no means a guarantee of crossing success. In *Nemesia*, we were trying to increase the range of flower colours in a couple of the perennial species by crossing them with the highly variable annual species. Although the genus has rather small chromosomes and the chromosome number and karyotype appear similar, we failed in our goal to generate hybrids between annuals and perennials. Crossing among perennials was possible and considerable variation was produced. After several rounds of selection perennial hybrids with greatly improved stature and range of flower colours was produced (Fig. 6A–D).

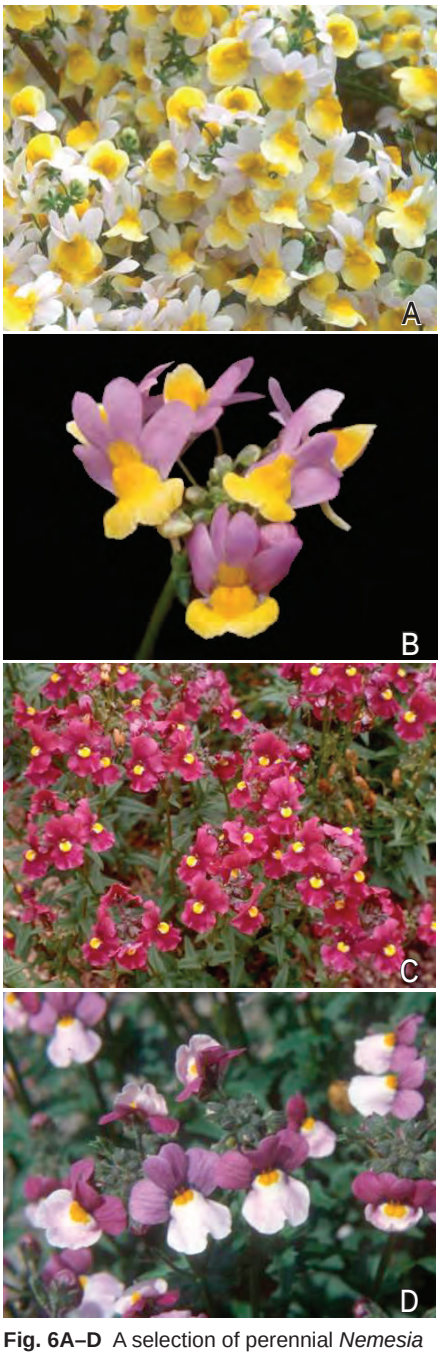

Fig. 6A–D A selection of perennial *Nemesia* hybrids showing the extensive range of variation obtained from crosses between perennial species.

Cytogenetics and hybrid identification

Several clivias have been suggested to be of hybrid origin, for example the 'Belgian' and 'German' hybrids and plants called *C. cyrtanthiflora.* This latter plant was suggested to be a hybrid between *C. miniata* and *C. nobilis* (Duncan, 1999), but there was no evidence that unambiguously identified its true origin (Bryan, 1995). The development of molecular cytogenetic techniques that use DNA to 'paint' or label chromosomes using DNA from whole species (genomic *in situ* hybridization, GISH) or known sequences of DNA (fluorescence *in situ* hybridization, FISH) has greatly

improved the analysis of karyotypes and the identification of genomes in hybrids. Details of the techniques can be found in manuals such as Schwarzacher and Heslop-Harrison (2000) but basically they involve the labelling of the chosen DNA probe with a tag that will allow its subsequent identification. The targets for the probe are the chromosomes prepared on a microscope slide. Both the probe and the target are denatured by heating to separate the two strands of their DNA molecules. The denatured probe and chromosomes are combined under controlled conditions that allow homologous sequences to 'find each other'. These *in situ* hybridization sites can then be identified by microscopic analysis.

By using these techniques we were able to establish that *C. cyrtanthiflora* was indeed a hybrid between *C. miniata* and *C. nobilis*. We used GISH, utilizing DNA extracted from the four known species, to probe the chromosomes of *C. cyrtanthiflora* and found that two of the species, *C. miniata* and *C. nobilis* gave clear hybridization patterns. Clivias have 22 chromosomes and 11 of these hybridized to the *C. miniata* probe and a different complement of 11 chromosomes hybridized to the *C. nobilis* probe (Fig. 7). These observations strongly suggest that *C. miniata* and *C. nobilis* are the parents of *C. cyrtanthiflora* and that these plants are the products of the initial cross or F_1 hybrids (Ran et al., 2001). Thus, the origin of *C. cyrtanthiflora* has been resolved and in a similar manner the 'Belgian' and 'German' hybrids were shown to be very similar to *C. miniata* and are not of interspecific hybrid origin.

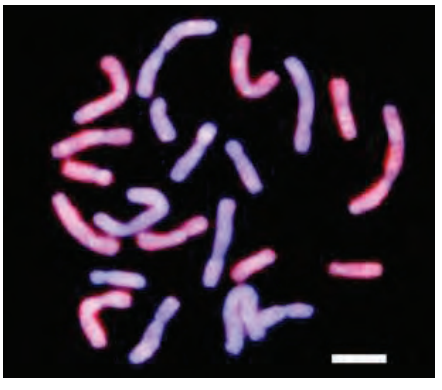

Fig. 7 Genomic *in situ* hybridization using DNA from *Clivia nobilis* onto the chromosomes of *C. cyrtanthiflora.* The chromosomes that have hybridized to the probe are pink and those that have not hybridized are blue. Scale bar = 10 µm.

Cytogenetics and species identification

In our studies of *Clivia* we found that although all the species have the same chromosome number and solid karyotype, the application of advanced techniques for chromosome analysis showed that there were small but significant and consistent differences in the karyotypes of the four initial species that we had for study. The distribution of different types of chromosome material that could be identified by chromosome banding techniques and the location of specific DNA sequences using FISH produced much more informative karyotypes than just the solid stained chromosomes. Thus, we were able to use the karyotype evidence to back up morphological studies to describe plants that were informally called "Robust gardenii" as a new species of *Clivia*, subsequently named formally as *C. robusta* (Murray et al., 2004). We have also been able to confirm that the sixth species of *Clivia*, *C. mirabilis* also has a unique karyotype (Murray et al., 2011; Fig. 8A–D).

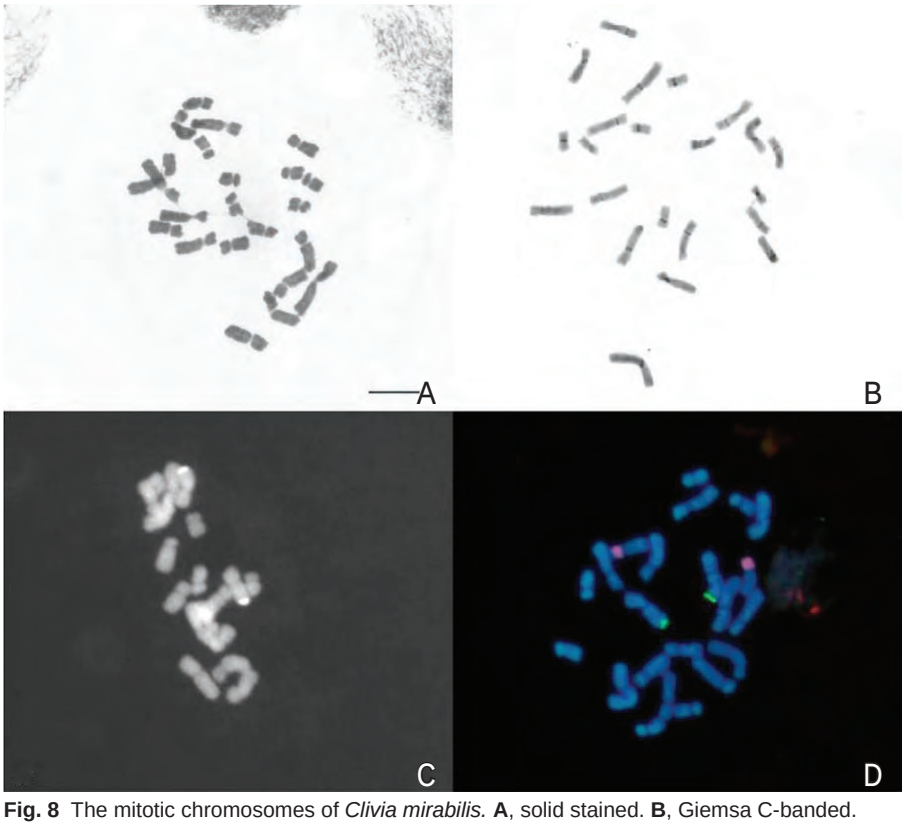

Fig. 8 The mitotic chromosomes of *Clivia mirabilis.* **A**, solid stained. **B**, Giemsa C-banded. **C**, fluorescent stained after treatment with chromomycin. **D**, FISH with a 45S rDNA probe (green) and a 5S rDNA probe (pink).

Conclusion

There is clearly no single technique or method to predict ease of hybridization between cultivated selections and their wild relatives – some can be crossed with ease and others with great difficulty or not at all. However, there is no doubt that some understanding of the cytogenetic makeup of parental materials can remove some of the guesswork and provide a scientific basis to ornamental plant breeding to extend the range of variation beyond that currently available.

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